REPRODUCTIVE POTENTIAL OF SNOW (*CHIONOECETES OPILIO*) AND TANNER (*CHIONOECETES BAIRDI*) CRABS IN ALASKA

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Abstract

Fisheries for snow (*Chionoecetes opilio*) and Tanner (*C. bairdi*) crab in Alaska are managed with large male only harvest regulations. Management of sex-selective crab fisheries could be enhanced by improved understanding of the functional relationship between male harvest and female reproductive potential. This research advances knowledge of factors associated with variation in reproductive potential by characterizing factors influencing female sperm reserves for Tanner crab, identifying factors associated with variability in fecundity for female snow crab in the eastern Bering Sea (EBS), and developing refined indices of egg (embryo) production and recruitment for snow crab that revealed a positive functional relationship that has not been previously described for this stock.

Sperm reserves of female Tanner crab varied with mature female ontogeny, sex ratio, and harvest. Increasing exploitation rate is associated with decreased average sperm reserves of primipara (first reproductive cycle) while increased availability of large, sexually-dominant, adult males, was associated with increased cumulative sperm reserves for multipara (second or greater reproductive cycle) among Tanner crab stocks. A white-layer of fresh ejaculate in the spermathecae (sperm-storage organ) was a robust indicator of increased sperm reserves in both primiparous and multiparous females and is likely a useful tool for evaluating risk of sperm limitation in *Chionoecetes.*

Fecundity of female snow crab in the EBS was influenced by both intrinsic and extrinsic factors. Fecundity increased with increasing female size and decreased for older multipara likely due to senescence. Variability in fecundity-at-size was higher among multipara than primipara and this may be associated with contrasting mating dynamics, tempo of reproduction, maternal age, or environmental influences on maternal condition. Mating success may also influence fecundity of multiparous females; females with fresh ejaculate had higher fecundity (~10%) than those that did not. Substantial embryo loss during brooding was not observed for snow crab, and embryo quality did not vary with female size or age relative to maturity.

Refining indices of female reproductive potential with demographic and fecundity information resulted in reduced estimates of reproductive output. A positive functional relationship between reproductive potential and recruitment was detected at a lag of four years due to coherence

between high reproductive output in the late 1980s and strong recruitment in the early 1990s. Stock productivity reached a minimum thereafter, preceding a rapid decline in mature abundance.

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General Introduction

Snow (*Chionoecetes opilio;* Fabricius, 1788) and Tanner (*C. bairdi;* Rathbun, 1924) crab (Brachyura: Oregoniidae) are distributed in high-latitude continental shelf habitats in the northern hemisphere. In Alaska, snow crab are found in the waters of the eastern Bering Sea (EBS) north of the Alaska Peninsula/Aleutian Islands and into the adjacent Artic Ocean, while Tanner crab occupy the Gulf of Alaska, Aleutian Islands, and the southern portion of the EBS. Both of these species have supported important commercial fisheries in Alaska waters with recent combined statewide average (2009-2013) annual harvests of ~67 million lb and an exvessel value of $110 million. However, harvests have fluctuated dramatically and current harvest levels are greatly reduced from years of peak harvests (e.g. 1990-1994) when combined average catch and value were 262 million lb and $222 million (nominal USD), respectively (Fitch et al. 2012). Moreover, steep declines in abundance and poor fishery performance in the mid to late 1990s led to large reductions in harvests and closures for EBS Tanner crab (Fitch et al. 2012). The EBS stocks of both snow and Tanner crabs were declared overfished under the US Magnuson-Stevens Fishery Conservation and Management and Conservation Act in October 1999 (64 FR 54791) and March 1999 (64 FR 15308), respectively. Overfished status requires stock rebuilding to a specified abundance level within ten years. The EBS snow and Tanner crab stocks were determined to be recovered from overfished status in 2011 (Turnock and Rugolo 2013) and 2012 (Stockhausen et al. 2013), respectively, but recent harvests and abundance remain at less than half that observed in the late-1980s to mid-1990s.

Maintenance of population reproductive potential is a primary management objective for Alaska crab stocks (ABOF 1990, NPFMC 2011,). In practice, large male-only harvest restrictions, along with catch quotas governed by a maximum fishing mortality rate to prevent overfishing, are implemented to achieve this management goal for the major EBS stocks. A challenging research objective for both Tanner and snow crab is to increase understanding of potential relationships between large male-only harvest and stock productivity or how varying rates of removal of large-males may impact female reproductive output. Lacking a robust index of female reproductive potential, mature male biomass is currently used as the index of reproductive potential for estimation of biological reference points for management of the major fisheries for both of these species. Improved understanding of two interrelated processes is needed to advance development

of refined indices of reproductive potential for both of these stocks. First, male-only exploitation may reduce fertilized embryo production by sperm limitation if insufficient males are present to adequately inseminate all the receptive females. Second, fertilized egg (embryo) production will vary interannually with abundance of mature females and the suite of density-dependent or density-independent factors which determine individual fecundity when sperm reserves are sufficient to maximize fertilized egg production.

Evaluation of the potential fishing impacts on stock reproductive potential requires knowledge of the complex life history and population dynamics of snow and Tanner crab, including early life history, growth, maturation, mortality, and recruitment variability. In Alaska, published descriptions of life history are more complete for female than male snow crab from the EBS and for male and female Tanner crab from the Gulf of Alaska (GOA) than from the EBS. Information is also available for snow crab stocks in Atlantic Canada, Russia and Japan. The duration of embryo incubation (oviposition to larval release) is approximately annual for Tanner crab and annual or biennial for snow crab (Sainte-Marie 1993, Swiney 2008). Ambient temperature strongly influences the duration of embryo incubation for snow crab; temperatures < 1° C prolong incubation from annual to biennial by triggering a 6 mo period of diapause early in embryo development (Kuhn and Choi 2011, Moriyasu and Lanteigne 1998, Webb et al. 2007). The duration of incubation is longer for primiparous than multiparous females of both species since primipara molt and undergo oviposition 1-4 mo earlier than multipara (Sainte-Marie 1993, Swiney 2008). Larval life history for both species consists of two zoeal stages and a single megalopal stage with a total larval duration of 3-4 mo for Tanner crab (Incze et al. 1987) and 2-4 months or more dependent on temperature for snow crab (Kogane et al. 2005, Kon et al. 2003, Yamamoto et al. 2014).

Male and female Tanner and snow crab have determinate growth, but growth schedules differ between species and between sexes within each species. Sexual size-dimorphism is observed in both species with terminally-molted (heretofore adult) males having larger average body size than females and adult Tanner crab of both sexes achieving larger body size than snow crab. Tanner crab reach larger sizes-at-age than snow crab by increased molt frequency during the first several years post-settlement. Tanner crab molt up to seven times during the first 2 yr post-settlement (Donaldson et al. 1981), while snow crab likely molt only four times (Alunno-Bruscia

and Sainte-Marie 1998, Comeau et al. 1998, Ernst et al. 2012, Sainte-Marie et al. 1995). Size-at-age relationships reflect this difference with Tanner crab of both sexes achieving substantially larger average size-at-age than snow crab (Alunno-Bruscia and Sainte-Marie 1998, Donaldson et al. 1981, Sainte-Marie et al. 1995). While juvenile growth rates are generally similar between sexes for each species, females transition to maturity at younger ages and smaller sizes than males, which tend to continue to grow for several more years prior to adulthood (Alunno-Bruscia and Sainte-Marie 1998, Donaldson et al. 1981, Sainte-Marie et al. 1995, Zheng 2008). Differences in maximum body size reflect these patterns with males exceptionally reaching ~ 200 mm and females ~ 120 mm carapace width (CW) for Tanner crab (Donaldson et al. 1981) and ~ 145 mm and ~80 mm respectively for male and female snow crab (Alunno-Bruscia and Sainte-Marie 1998, Orensanz et al. 2007, Sainte-Marie et al. 1995, Turnock and Rugolo 2012). Growth patterns are modulated by environmental factors, primarily temperature, for snow crab and likely Tanner crab. Differences in ambient temperature through ontogeny influence molt frequency during the first few years after settlement and later in ontogeny (skip-molting) in larger immature males and females (Burmeister and Sainte-Marie 2010, Dawe et al. 2012, Orensanz et al. 2007). Molt frequency is hypothesized to decrease with decreasing temperature leading to reduced size-at-maturity but similar age at maturity for individuals of both sexes growing under colder versus warmer conditions for snow crab (Burmeister and Sainte-Marie 2010, Ernst et al. 2012, Orensanz et al. 2007, but see Dawe et al. 2012). Similar patterns may explain observations of regional differences in male and female size-at-maturity for Tanner crab but this hypothesis has not been comprehensively evaluated (Siddon and Bednarski 2010, Somerton 1981, Zheng 2008).

Transitions to functional maturity with increasing size/age are less complex for female versus male snow and Tanner crabs. Allometric growth of male chelae and female abdomen sizes relative to CW are indicators of completion of the terminal molt to maturity for both snow and Tanner crabs (Conan and Comeau 1986, Hilsinger 1976, Tamone et al. 2007, Watson 1970). Based on size and color, onset of ovarian maturation in juvenile female Tanner crab is first detectable at 40-50 mm CW, but some females up to 60-70 mm CW do not have maturing ovaries (Donaldson 198, Hilsinger 1976,). Onset of ovarian maturation for female snow crab in eastern Canada occurs at 20-30 mm CW and most females > ~30 mm CW have maturing ovaries (Alunno-Bruscia and Sainte-Marie 1998). Adult snow and Tanner crab of both sexes have broad

size-frequency distributions. Adult female Tanner crab range in size from ~60 to 100 mm CW, but most are primarily between 80 and 95 mm CW in the GOA (Donaldson et al. 1981, Hilsinger 1976). Likewise, female snow crab in the EBS range in size from ~40 mm to 80 mm CW, but sizes of 50 to 70 mm CW are predominant with a trend towards reduced female size with increasing latitude (Orensanz et al. 2007, Somerton 1981). Male snow and Tanner crab produce spermatophores over a considerable size range (termed adolescent) and can mate successfully, but larger body size and completion of the terminal molt to adulthood are precursors to full functional maturity. Spermatophores are present in the vas deferens of male Tanner crab as small as ~40 mm CW (Paul and Paul 1990), but mating success is low at this size and increases with increasing male size up to 85 mm CW (Paul and Paul 1990). Patterns of male gonadosomatic index and chelae height as a function of CW indicate larger sizes-at-maturity of ~110 mm CW in the GOA (Brown and Powell 1972) and ~115 mm CW in the EBS (Somerton 1980). The size-range of adult male Tanner crab extends from ~80 to 180 mm CW for Tanner crab in both the GOA and the EBS (Donaldson et al. 1981, Zheng 2008). Finally, direct observations of grasping pairs at the time of mating indicate that terminal molt status is an indicator of functional maturity for Tanner crab. The mean CW of males observed grasping females during the mating period was 114 mm CW and >99% of individuals found grasping pubescent and primiparous/multiparous females were terminally-molted, large-claw males (Stevens et al. 1993). Patterns of maturity with size are similar for snow crab with spermatophores present in 50% of males at 38 mm CW (Sainte-Marie et al. 1995), increasing reproductive potential with increasing male size for adolescent males (Sainte-Marie et al. 1995), and functional maturity of large, adult males as the primary participants in mating *in situ* versus adolescent males (Comeau et al. 1998, Moriyasu and Comeau 1996). Importantly, both adolescent and small adult males can successfully mate with both primiparous and multiparous females and do actively participate in mating in the absence of adult males (Sainte-Marie et al. 2008, Somerton 1981).

For snow and Tanner crabs and crustaceans in general, the presumed lack of structures retained throughout the lifetime due to molting has hindered the development of comprehensive information on age structure, which would be useful to estimate parameters related to survival including both longevity and natural mortality. In the absence of direct aging techniques, indirect methods including analysis of size-frequency distributions, growth, mark-recapture experiments, and more recently the accumulation of the metabolic pigment lipofucsin in neural tissue have

been the primary methods used to investigate and infer age structure (Hartnoll 2001, reviewed by Vogt 2012). With validation, a recently proposed novel direct aging method for decapod crustaceans may result in improved age estimates and life history information (Kilada et al. 2012). For snow and Tanner crab, classification of individuals by condition of exoskeleton, based on gradients in shell hardness, color, wear, and accumulation of epibionts, has proven useful as a rough index of determining time-elapsed relative to the terminal molt to maturity for both males and females. In Alaska, individuals are subjectively assigned to four or five shell condition classes ranging from soft/molting to very, very oldshell (Jadamec et al. 1999) during fishery-independent pot and trawl surveys. The system of classification varies between agencies and surveys with four classes used in the Alaska Department of Fish and Game Southeast Alaska Tanner crab pot survey and applied in chapter 1 of this dissertation and five classes used during the National Marine Fisheries Service eastern Bering Sea crab and groundfish trawl survey and applied in chapters 2 and 3 of this dissertation. While shell condition index data may have limited accuracy and precision as a method of determining age of an individual, its general usefulness has been confirmed for male and female snow crab by tag-recapture studies, radiometric analysis, and cohort abundance dynamics (Ernst et al. 2005, Ernst et al. 2012, Fonseca et al. 2008). From these studies, as well as studies of growth from the first benthic stages to maturity, lifespans of approximately 18 yr for males (Fonseca et al. 2008, Sainte-Marie et al. 1995) and 14 yr for females (Ernst et al. 2005, Ernst et al. 2012) have been inferred for snow crab. Similar studies have not been conducted for Tanner crab. Shell condition has also been used as a factor in the estimation of natural mortality rates for snow crab. Natural mortality rates for mature female snow crab were estimated from stage-based models structured by shell condition for EBS (Zheng 2003) and by annual variation in size-at-maturity and abundance by shell condition in eastern Canada (Drouineau et al. 2013).

Highly variable recruitment is characteristic of snow and Tanner crab (Zheng and Kruse 2000, Zheng and Kruse 2006). Hypotheses regarding density-dependent and density-independent factors influencing recruitment variability have been developed for both snow (Kruse et al. 2007, Orensanz et al. 2004, Sainte-Marie et al. 1996) and Tanner crab (Tyler and Kruse 1997). The EBS snow crab stock has been the focus of intensive investigation in the past decade. Key findings of these studies include strong cyclic patterns of abundance for males and females (Parada et al. 2010), unidirectional migration from shallower to deeper waters with ontogeny for

mature females (Ernst et al. 2005), metapopulation structure due to variation in the estimated probabilities of larval retention and connectivity across the distribution (Parada et al. 2010), key role of primiparous females on stock renewal dynamics (Burgos et al. 2013, Parada et al. 2010), spatiotemporal shifts in predation mortality of juvenile snow crab by Pacific cod (*Gadus macrocephalus*, Burgos et al. 2013), stronger recruitment associated with colder bottom temperatures (Marcello et al. 2012), and a shift in recruitment drivers from parental reproductive output to the Pacific Decadal Oscillation (Szuwalski and Punt 2013), an index of climate variability in the North Pacific (Mantua et al. 1997). Drivers of recruitment variability were also evaluated for the Bristol Bay portion of the Tanner crab stock in the EBS. Recruitment was positively associated with prevailing wind direction hypothesized to be favorable for advection of larval transport to nearshore nursery areas and negatively associated with cold bottom temperatures which could unfavorably impact female reproduction (Rosenkranz et al. 2001). Some synchrony was found in recruitment variation among Tanner crab in the Gulf of Alaska but recruitment dynamics were not broadly associated with any of the environmental variables examined (Zheng and Kruse 2000).

Sperm limitation may be the primary mechanism by which recruitment overfishing can occur in crab stocks with male-only harvest rules, but it is has proven difficult to discriminate between the roles of fishing and environmental variability in the dynamics of reproductive potential and recruitment in Alaskan crab stocks (Orensanz et al. 1998*).* Reductions in fertilized egg production due to sperm limitation were demonstrated or hypothesized for commercially important brachyuran and lithodid crab stocks including *Callinectes sapidus* (Carver et al. 2005, Hines et al. 2003), *Metacarcinus magister* (Smith and Jamieson 1991), snow crab (Rondeau and Sainte-Marie 2001, Sainte-Marie et al. 2002), *Paralithodes brevipes* (Sato et al. 2007), and *Paralithodes camtschaticus* (McMullen 1968, Orensanz et al. 1998). These studies provide evidence that the probability of sperm limitation increases both as the sex ratio at the time of mating becomes skewed towards females and with variability in the characteristics of males available for mating. Small and recently molted males frequently have reduced fecundity relative to larger males and males that have not recently molted. Risk of sperm limitation is also mediated by behavior including competition for mating opportunities among dominant phenotypes, preference of large individuals to mate with other large individuals, and demonstrated ability for some species to detect and avoid mating with individuals with low

reproductive potential. Biological, environmental, and anthropogenic factors associated with risk of sperm limitation for brachyuran and lithodid crabs are reviewed by Sainte-Marie (2007) and Sato (2012) and for snow crab in particular by Sainte-Marie et al. (2008).

Monitoring of female mating success is recommended, in addition to indices of reproductive potential and recruitment, for the management of exploited crustacean stocks (Smith and Sainte-Marie 2004). Development of indices of female reproductive potential and associated biological reference points for snow and Tanner crab have been hampered by limited understanding about how varying large-male harvest rates impact female reproductive potential and, particularly for snow crab, how female demography, ontogeny, and temperature-induced biennial reproduction affect reproductive potential. Internal storage of sperm is widespread in brachyuran crab and quantifying the contents of the spermathecae (female sperm storage organ) can provide direct information on variation in female mating history and success (e.g. Jivoff 1997, Sainte-Marie et al. 2002, Xuan et al. 2014). In Alaska, monitoring of female sperm reserves has been conducted for snow and Tanner crab in an effort to improve understanding of factors associated with interannual variability in reproductive potential (Gravel and Pengilly 2007, Slater et al. 2010). However, this information is not formally considered in the stock assessment process for exploited stocks of snow and Tanner crab in Alaska. Stocks of both species are typically monitored annually with fishery-independent stock assessment surveys, which, with some limitations, provide information on the relative abundance, size-frequency, size, maturity, and distribution of males and females. These datasets can be used to estimate reproductive parameters, such as sex ratio which are known to influence female reproductive success. Nevertheless, progress in using this information has been limited by a lack of sufficient biological data on sperm reserves and fertilized egg production collected at appropriate spatiotemporal scales to identify conditions which indicate a risk of reduced reproductive potential.

Available information on variation in female fecundity of snow crab and Tanner crabs in the North Pacific Ocean is limited to a few prior studies, which found greater fecundity at size for Tanner crab than snow crab (Haynes and et al. 1976, Jewett 1981). Multiparous female Tanner crab also have higher fecundity-at-size than primiparous Tanner crab (Somerton and Meyers 1983), but studies for snow crab either sampled only primipara (Haynes and Karinen 1976) or

did not differentiate between these groups (Jewett 1981). More recent studies focused on variation between female size, clutch dry weight, and development of indices of "clutch fullness", which are visually assigned for snow and Tanner crab during fishery independent surveys (Orensanz et al. 2005) and were used to investigate variability in female reproductive potential relative to maximum clutch weight at size for snow crab (Armstrong et al. 2008). Development of egg production indices requires quantitative descriptions of the functional relationship between density-dependent and density-independent variables and the size-fecundity relationship, preferably near the time of larval release. Finally, time-series of estimated female abundance, demography, and for snow crab the spatial distribution of females in relation to bottom temperatures likely to prompt biennial reproduction are needed for integration with fecundity information to estimate egg production as an alternative index of reproductive potential.

The goal of this dissertation is to improve understanding of the role of exploitation in influencing variability in reproductive potential for snow and Tanner crab stocks by completing three primary objectives: (1) to advance understanding of the influence of ontogeny, sex ratio, and harvest rate on female sperm reserves and to evaluate indicators of mating useful for field assessment of female mating success among six discrete stocks of Tanner crab in Southeast Alaska (Chapter 1), (2) to characterize factors impacting female fecundity, including demography, embryo loss during brooding, and possible variation in embryo quality for eastern Bering Sea snow crab (Chapter 2), and (3) to develop indices of egg production that integrate information on fecundity, female abundance and demography, and biennial reproduction as an alternative index of reproductive potential for EBS snow crab. Variation in egg production indices are compared to indices of reproductive potential based on mature abundance and examined for functional relationships to recruitment (Chapter 3).

Chapter 1

Variability in reproductive potential among exploited stocks of Tanner crab, *Chionoecetes bairdi*, in southeastern Alaska[1]

Abstract

For many exploited crab stocks, management has been challenged by uncertainty in estimation of abundance, effective spawning biomass, and stock-recruit relationships. Crab fishery management strategies strive to optimize tradeoffs between yield and variability in yield by harvesting males only above a minimum size limit, typically set at one molt larger than size of 50% male maturity. However there is limited understanding of the implications of this harvest strategy for mating frequency and female reproductive potential (e.g. sperm limitation). To evaluate reproductive potential of female Tanner crab (*Chionoecetes bairdi*), we characterized egg viability, spermathecal load, and sperm cell counts in six spatially discrete locations in southeastern Alaska. All females carried viable egg clutches with low incidence ($\bar{x}$ <1%) of non-viable eggs. Mean spermathecal load was significantly lower in primiparous compared to multiparous females in four of five locations compared, but sperm cell counts were similar between primiparous and multiparous females in all locations. Mean sperm cell counts were significantly higher when fresh ejaculate was present versus when it was absent for both primiparous (454% higher) and multiparous females (320% higher). The proportion of primiparous and multiparous females with fresh ejaculate was correlated with mean sperm cell counts by location and may be a useful index of variability in female sperm cell counts among stocks. Evidence of sperm limitation was not observed in this study, but mean sperm cell counts of primiparous females by location were negatively correlated with exploitation rate index suggesting that male-only harvest may decrease levels of stored sperm available for fertilization of a subsequent clutch.

[1] Webb, J.B., and J. Bednarski. 2010. Variability in reproductive potential among exploited stocks of Tanner crab (*Chionoecetes bairdi*) in southeastern Alaska. *In* Management of expoited crab population under climate change. *Edited by* G.H. Kruse, G.L. Eckert, R.J. Foy, R.N. Lipcius, B. Sainte-Marie, D.L. Stram and D. Woodby. Alaska Sea Grant College Program AK-SG-10-01, University of Alaska Fairbanks. pp. 295-317.

1.1 Introduction

Management of many decapod crustacean fisheries is complicated by lack of precise aging techniques, high recruitment variability, and uncertainty in stock-recruit relationships, all of which can result in difficulty in estimation of stock productivity and sustainable harvest levels. In response to this uncertainty, conservation and monitoring of population reproductive potential have been recommended (Kruse 1993, Smith and Sainte-Marie 2004) and adopted as a primary management objective for crab stocks in Alaska (NPFMC 1998, Woodby et al. 2005).

A management concern for exploited crab stocks is that size- and sex- selective exploitation or natural variability in sex ratio may result in decreased population egg production due to sperm limitation (Orensanz et al. 1998). Specifically, female skewed sex-ratios associated with large-male only harvest or natural variability could result in insufficient availability of males with the characteristics necessary to fully inseminate and maximize the viable egg production of all mature females (Hines et al. 2003, Sato et al. 2007, Smith and Jamieson 1991). Recent contributions have improved understanding of crab life history, reproductive biology, and mating systems (e.g. Sato et al. 2005, Sainte-Marie et al. 2008) and the robustness of biological indicators of recent mating for females has been evaluated for at least one species (Duluc et al. 2005). However, progress in integrating this knowledge into development of indices of female mating success and sperm reserves at the appropriate spatial and temporal scales for management has been limited.

Evaluation of multiple factors including fecundity, egg viability, and sperm reserves are likely to be necessary for characterization of variability in reproductive potential. For Tanner *(Chionoecetes bairdi)* and snow *(C. opilio)* crabs, commercially valuable species in Alaska, reductions in viable egg production due to sperm limitation are likely to be a threshold response (Paul and Adams 1984, Sainte-Marie and Lovrich 1994, Sainte-Marie et al. 2002); and may be observed as occurrences of barren mature females, females with clutches of mixed viable and non-viable eggs, or reduced fecundity if non-viable eggs are lost during brooding (Paul 1984, Sainte-Marie and Carriere 1995). For Tanner and snow crabs, species that store sperm across annual or biennial reproductive cycles, monitoring of egg viability and fecundity could provide information on spatiotemporal variability in viable egg production. Estimates of female sperm

reserves could be used to assess mating frequency and the relative spatiotemporal vulnerability of crab stocks to sperm limitation.

The Tanner crab is a commercially valuable species in Alaska targeted by commercial fisheries from the eastern Bering Sea to southeast Alaska. Statewide annual harvests peaked in the early 1980s at about 82 thousand metric tons before declining precipitously and continuing at low levels (~4.5 thousand metric tons) with intermittent or long-term closures in all management areas through the present (Woodby et al. 2005). Key features of Tanner crab reproductive biology include a terminal molt to maturity for both sexes and complex mating behaviors and dynamics. Female Tanner crab that complete the terminal molt to reproductive maturity, after which copulation and extrusion of the first egg clutch occurs, are termed primiparous (Paul and Adams 1984). Multiparous females, carrying a second or later clutch, may fertilize a clutch from fresh sperm obtained by mating in hard-shell condition after larval release or stored sperm from previous matings (Adams and Paul 1983). After mating, a new clutch of eggs is extruded onto the female's abdomen and incubated for approximately twelve months until hatching (Donaldson and Adams 1989, Swiney 2008, Webb 2009).

A fishery for Tanner crab has occurred in southeastern Alaska, a complex network of fjords, islands, and connecting waterways, since the 1960s. Annual stock assessment surveys have been conducted in six spatially discrete locations in southeast Alaska since 2001 (Bednarski et al. 2008). Legal and mature male biomasses are independently estimated annually for each location from a two-stage catch-survey analysis model (Zheng et al. 2006). Model estimates of male biomass and survey indices of the male:female sex ratio contrast among locations. These data provide a framework to assess functional relationships between these indices and direct measures of reproductive potential including the prevalence of indicators of recent mating, viable egg production, and sperm reserves. The objectives of this study were to assess the fecundity, egg viability, and sperm reserves among primiparous and multiparous female Tanner crab from the survey areas in southeast Alaska, compare these measures with survey and model indices of sex ratio and exploitation rate, and evaluate indicators of recent mating as robust indicators of sperm reserves.

1.2 Materials and Methods

Female Tanner crab were collected from six locations during the Alaska Department Fish and Game southeast Alaska Tanner crab pot survey in October of 2007 (Figure 1.1). Pot survey design and survey biological data collection protocols are detailed in Bednarski et al. (2008). Female Tanner crab were haphazardly collected from pot catches to meet sampling goals by shell condition (n=15 females for each shell condition class) within each survey location. Females were classified by shell condition, which is an assessment of color and wear on the exoskeleton, as new shell (SC3), old shell (SC4), or very old shell (SC5) (Jadamec et al. 1999). For clarity, new shell, primiparous females with clean firm exoskeletons brooding their first egg clutch; multiparous females with exoskeletons of intermediate shell condition, likely brooding their second or third egg clutch; and multiparous females with darkened, highly worn exoskeletons, which are likely brooding their third or greater clutch of ontogeny will be referred to as SC3, SC4, and SC5, respectively. Sample sizes were limited in some locations due to limited catches of females of certain shell conditions. Upon collection females were individually marked with numbered plastic tags attached to the third left periopod using a plastic tie. In Holkham Bay, Thomas Bay, and Port Camden females were dissected aboard the vessel. The abdomen and egg clutch of each female was detached and preserved in 80% ethanol and the right spermatheca was removed without perforation and preserved in 10% formalin. In Stephens Passage, Icy Strait, and Glacier Bay females were frozen and processed for reproductive condition in the laboratory.

1.2.1 Indicators of mating and sperm reserves

Females were assessed at the time of dissection for indicators of mating including fresh grasping marks (Paul 1984) and the presence of fresh ejaculate at the open (ventral) end of the spermathecae (Duluc et al. 2005). Fresh grasping marks visible as areas of lighter coloration on the dorsal surface of the first three walking legs are due to abrasion of the exocuticle by the chelae of males as females are grasped during the mating period. Pubescent/primiparous females are unlikely to have grasping marks after mating due to the soft condition of the exoskeleton during the molting/mating period.

For measurement of sperm reserves, the right spermatheca was removed from formalin at least seven days after preservation. The wall of the spermatheca was removed and the spermathecal load (SL) or weight of the spermathecal contents was measured to the nearest 0.1 mg on a

Mettler AE163 analytical microbalance. Estimated sperm cell counts were determined by homogenization and dilution of spermathecal contents followed by visual counts of sperm cells using replicate hemacytometers. The estimated number of sperm cells in the spermatheca was then calculated as the mean of four replicate counts multiplied by the dilution factor. All sperm cell counts were those of the right spermatheca only and total sperm counts per female would be approximately double assuming similarity of sperm cell counts between the paired spermathecae (Sainte-Marie and Lovrich 1994).

1.2.2 Indices of sex ratio and harvest rate

To evaluate variability in reproductive potential with indices of sex ratio among locations, mean pot catch-per-unit-effort (CPUE) was calculated for various combinations of female and male Tanner crab by size and shell condition from the survey conducted in October 2007 (Table 1.1). Survey sampling design, gear, and biological data collection protocols are detailed in Bednarski et al. (2008). Escape mechanisms are blocked on survey pots to increase retention rates for small crab; females at least one molt prior to the molt to maturity are commonly observed in survey sampling, indicating that pots retain female crab at or below the size-at-maturity (~80 mm carapace width, Hilsinger 1976). A second alternative index of sex ratio was also calculated by location as the ratio of estimates of legal and total mature male biomass from a catch-survey-analysis model (Zheng et al. 2006) to multiparous and primiparous female survey CPUE. To assess possible relationships between harvest and sperm reserves an index of exploitation rates by location was determined for each survey location from the model estimates of legal male biomass divided by the landed catch in number of individuals in the subsequent winter fishery (February 2007).

1.2.3 Egg viability and fecundity

To determine the proportion of viable eggs in a clutch, two subsamples of ~200 to 250 eggs were removed from the center and periphery of the dorsal surface of the egg clutch and individual eggs were classified as viable or non-viable based on structure and color. Eggs were primarily in the intermediate, yolk reduction stage of development allowing reasonable determination of viable versus non-viable eggs from ethanol preserved and frozen samples. For measurement of fecundity, the replicate subsamples examined for viability were counted and placed in pre-weighed drying pans. The remaining eggs in the clutch were then gently stripped from the

pleopods using forceps and placed in a pre-weighed drying pan. Embryo subsamples and clutch were dried to constant weight in a 60° C oven for > 48 h and weighed to the nearest 0.1 mg on a Mettler AE163 analytical microbalance. Fecundity was estimated by dividing the clutch dry weight by the mean of the weight of an individual embryo estimated from the two counted subsamples. Differences in preservation method were included as a factor in statistical analyses to account for possible differences in fecundity estimation with preservation method.

1.2.4 Statistical methods

Nested analysis of variance was used to evaluate differences in sperm reserves by shell condition among locations. Levene's test for homogeneity of variance, residual plots, Cook's distance, and the Shapiro-Wilk test for normality were used to assure that data met assumptions for analysis. Both spermathecal load and sperm cell count data met assumptions after Box-Cox transformation and significant ($p< 0.05$) differences between groups were determined using a Tukey HSD post-hoc test. When influential outliers were detected, the model was refit excluding the outliers and differences between group means were examined for changes in significant differences. Differences in sperm cell counts and spermathecal load with the presence or absence of indicators of recent mating within shell condition groups were examined with Mann-Whitney/Wilcoxon rank sign tests. The Bonferroni adjustment was used to increase the threshold of significance (α) to control for Type I error due to multiple pair wise comparisons. Relationships between sperm reserves, survey and model indices of sex ratio, and exploitation rate index were compared using Pearson correlation coefficients. Analysis of covariance was used to examine sources of variability in fecundity with main factors of carapace width, shell condition, mean embryo weight, clutch preservation method, and location. Non-significant terms ($p > 0.05$), which included location, preservation method, and an interaction term between these variables, were dropped from the model. Statistical analyses were conducted in JMP 8.02 (SAS Institute, Cary, NC).

1.3 Results

1.3.1 Sperm reserves by shell condition and location

The number of primiparous and multiparous female Tanner crab collected and processed for sperm reserves varied among survey locations (Table 1.2). For females from all locations

combined, a linear relationship was observed between sperm cell count and spermathecal load in SC3 females ($r^2 = 0.67$, $F = 119.7$, $p < 0.0001$, d.f. = 59), but not for SC4 or SC5 females for which a reduced range of spermathecal load values was observed (Figure 1.2). Spermathecal load varied significantly ($R^2=0.63$, $F=16.82$, d.f.=131, $p<0.0001$) among locations and shell condition groups. Spermathecal load was higher for SC4 and SC5 versus SC3 females in four of five locations and similar between SC4 and SC5 multiparous females in the locations in which SC5 females were sampled (Table 1.2). Within the SC3 (primiparous) shell condition category, females from Port Camden, Holkham Bay, Thomas Bay and Stephens Passage had greater spermathecal load than those in Glacier Bay or Icy Strait. While for SC4 and SC5 (multiparous) categories, females in Thomas Bay had lower spermathecal load than those from the other locations (Table 2). Within location, with the exception of Thomas Bay, SC4 and SC5 females had significantly greater spermathecal load than SC3 females (Table 1.3). SC4 females from Port Camden were excluded from comparisons of sperm reserves by location and shell condition due to low sample sizes.

Sperm cell counts also varied significantly ($R^2 = 0.22$, $F = 2.82$, d.f. = 131, $p < 0.002$) among groups by shell condition and location, but few significant differences were observed relative to the number of groups compared and the model explained a low proportion of the overall variance observed. SC3 females in Holkham Bay had significantly greater sperm cell counts than those in Glacier Bay and Icy Strait, and SC4 females from Stephens Passage had greater sperm cell counts than SC3 females in Icy Strait (Table 1.2). Mean sperm cell counts were generally similar among shell condition groups within a location, and no significant differences in sperm cell counts were observed among shell condition groups within locations (Table 1.2).

1.3.2 Indicators of recent mating

The presence of fresh ejaculate or a white deposit at the ventral, open end of the spermathecae for all females and fresh grasping marks on the anterior pairs of periopods for multiparous females have been proposed as indicators of recent mating and were associated with increased sperm reserves in this study. Considering females from all locations combined, spermathecal load was significantly higher ($Z = 5.02$, $p < 0.0001$) in SC3 females classified as having fresh ejaculate versus those without, but a similar result was not observed for SC4 ($Z=1.23$, $p = 0.22$) or SC5 ($U = 0.46$, $p = 0.52$) females (Table 1.3). Estimated mean sperm cell counts were

significantly higher for SC3 (Z =4.90, $p < 0.0001$) and SC4 females (Z = 2.61, p = 0.0086) with versus without fresh ejaculate, but did not differ for SC5 (U = 4.12, d.f. = 1, p = 0.042) females (Table 1.3). For SC4 females the presence of fresh grasping marks was associated with significantly higher sperm cell counts (Z = 2.19, p = 0.028), while spermathecal load was similar between groups (U = 0.57, p = 0.56). Higher sperm cell counts with the presence of fresh ejaculate suggested that an increase in mean sperm cell count might be observed with an increased proportion of females with indicators of recent mating in each study location. This was confirmed as a significant positive correlation (r = 0.91, d.f. = 5, p = 0.011) observed between mean sperm cell counts for SC3 females by location and the proportion of SC3 females in each location classified as having fresh ejaculate (Table 1.4, Figure 1.3). Similarly, a significant positive correlation (r = 0.78, d.f. = 12, p = 0.002) was observed between the mean sperm cell counts of females of all shell condition groups by location and the proportion of females with fresh ejaculate (Table1.4, Figure 1.3).

1.3.3 Indices of sex ratio and exploitation rate

Results of correlations between sperm reserves, sex ratio indices, and exploitation rate index by locations were mixed (Table 1.5). A significant negative correlation was observed between the mean spermathecal load of SC3 females and the index of exploitation rate by location (Table 1.5, Figure 1.4). The strongest correlations observed for multiparous females were between model estimates and survey indices of male abundance to the survey index (CPUE) of SC4 and SC5 female abundance (Table 1.5, Figure 1.4). This pattern may imply that the mean spermathecal load of multiparous females may increase with increased abundance of legal–sized males relative to SC4 and SC5 females among locations.

1.3.4 Egg viability and fecundity

Female carapace width, shell condition, and mean embryo weight were significant predictors of variability in female fecundity (ANCOVA, Table 1.6), while preservation method and location were not. Non-significant effects were removed and the reduced model was used to estimate parameters. The reduced model fit the data well (r^2=0.86, F = 193.2, $p < 0.0001$) and significant differences were observed for each level of the shell condition factor. Estimated from ANCOVA adjusted means, SC4 females ($\bar{Y}$ = 190,373) were 48% more fecund than SC3 ($\bar{Y}$ = 116,021) females and the fecundity of SC5 ($\bar{Y}$ = 156,546) females was intermediate, 30% higher than SC3

females but 20% lower than SC4 females. The proportion of non-viable embryos and egg cases observed in subsamples from the clutches of SC3 and SC4 females was less than 5% across locations (Figure 1.5). The mean proportion of non-viable embryos was less than 1.0% in all locations (Figure 1.6), and the mean proportion of egg cases ranged from a minimum of 0.39% for SC3 in Glacier Bay to a maximum of 2.9% for SC3 females in Thomas Bay. No trends of or thresholds for reductions in fecundity were observed in comparisons of residuals of predicted fecundity (observed - predicted) with sperm cell counts, indicating that sperm limitation was unlikely in this study (Figure 1.6).

1.4 Discussion

Characterization of the patterns of sperm reserves by location and between females of varied reproductive history (e.g. primiparous versus multiparous) may provide insight into variability in sperm reserves and, by extension, resilience against future sperm limitation. In this study we observed differences in spermathecal load but similar sperm cell counts between primiparous and multiparous females. This difference may have been due to the accumulation of seminal plasma, transferred with spermatophores during mating (Sainte-Marie and Sainte-Marie 2000), as the duration of an individual's reproductive history increased. Hypothetically, if females mated on an annual basis but a high proportion of the sperm cells acquired at mating were used for egg fertilization then seminal plasma could accumulate without a concurrent increase in the number of stored sperm cells. In support of this hypothesis, a very similar pattern was observed for primiparous and multiparous female snow crab from Japan (Yamasaki 1994; their groups A and B). Factors determining patterns of sperm cell counts and spermathecal load are likely to differ between primiparous and multiparous female Tanner crab. Primiparous females generally molt to maturity and mate in shallow water from January to May (Stevens et al. 1994), whereas multiparous females hatch their larvae and mate over several weeks in April and May (Stevens 2003, Webb 2009). The extended duration of the timing of the molt to maturity and sperm storage from a single mating period for primiparous females versus a temporally compressed mating period and sperm storage reflecting integration of multiple annual reproductive cycles in multiparous females could result in contrasting patterns of sperm reserves between these groups. Our results indicated that female Tanner crab in southeast Alaska were unlikely to accrue significantly greater sperm reserves in terms of number of sperm cells with increasing duration of reproductive history.

Improved understanding of patterns of female sperm storage among stocks may assist in characterization of the expected range of variability in sperm reserves among stocks and in identifying stocks with greater vulnerability to sperm limitation. Among the stocks evaluated in this study, SC4 females in Holkham Bay and Stephens Passage had greater spermathecal load than those in Thomas Bay (Table 2). Assuming a similar number of reproductive cycles completed among SC4 females across locations; females in Holkham and Stephens may have, through ontogeny, experienced mating conditions (e.g. sex ratio) more favorable to acquisition of sperm reserves than those in Thomas Bay. Consistent with observations for SC4 females, SC3 females from Holkham Bay had greater mean sperm cell counts than females from Glacier Bay or Icy Strait (Table 2). These results suggest that females in Holkham Bay may be more likely to acquire greater sperm reserves perhaps due to greater availability of males during mating than other locations in southeastern Alaska.

Field studies of female Tanner crab sperm reserves in Alaska have only recently been undertaken and have differing results. The mean spermathecal load of primiparous (SC3) females in this study (0.0385 g ± 0.0060 g) was lower than those observed for primiparous females (0.0895 g ± 0.013 g) from the southeastern Bering Sea. However, mean sperm cell counts were higher (1.98 X 10^7 ± 3.14 X 10^6 vs. 5.54 X10^6 ± 1.30 X 10^6) for females in this study (Gravel and Pengilly 2007). Mean spermathecal loads and sperm cell counts of SC4 females in this study (0.20 ± 0.016 g, 2.32 X 10^7 ± 3.52 X 10^6) were lower than multiparous females from southeastern Alaska inferred to have recently mated (0.44 g ± 0.026 g, 9.35 X 10^7 ± 7.83 X 10^6) *in situ*, but similar to those which fertilized a clutch with stored sperm without access to males (0.21 g, ± 0.043 g, 2.00 X 10^7 ± 7.53 X 10^6) in the laboratory (Webb 2009).

Both grasping marks and the presence of fresh ejaculate in the spermatheca have been used for estimating variability in the frequency of recent mating for snow crab (Taylor 1996, Duluc et al. 2005). In this study the presence of a white layer of fresh ejaculate at the base of the right spermatheca indicated a highly significant increase (a factor of 2.96) in spermathecal load and sperm cell count (a factor of 4.54) for SC3 females and sperm cell counts for SC4 females (a factor of 3.20) (Table 3). The presence of fresh grasping marks was also associated with an increase in sperm cell counts for SC4 females (a factor of 1.92). The larger relative difference in sperm cell counts based on fresh ejaculate versus fresh grasping marks is similar to the

experimental findings of Duluc et al. (2005), which identified fresh ejaculate as an improved indicator of recent mating. In this study all SC3 females carried clutches of fertilized eggs at the time of collection and had likely acquired adequate sperm reserves for clutch fertilization during mating at the molt-to-maturity or shortly thereafter. Thus, for SC3 females, the presence of fresh ejaculate was useful for classification of females with greater or lesser levels of sperm reserves stored from mating at the molt- to-maturity. The spermathecae of multiparous females with visible fresh ejaculate often contained one or more ejaculates darker in color (beige to black) located dorsally of the white ejaculate. If the entire deposit of fresh ejaculate was expended for clutch fertilization and the spermathecae contained only darker ejaculate, it is likely that the female would not be recognized as recently mated (Duluc et al. 2005). Thus, similar to primiparous females, the presence of fresh ejaculate in the spermathecae of multiparous females is indicative of females with sperm reserves acquired during recent mating in excess of that needed for clutch fertilization. The difference in mean spermathecal loads (0.04 g) and sperm cell counts ($1.98 \text{ X } 10^7$) observed between multiparous females with or without ejaculate in this study was less than those (0.24 g, $7.35 \text{ X } 10^7$) observed between females, which had fertilized a clutch with stored sperm or likely mated and extruded a clutch fertilized with sperm from recent mating (Webb 2009). Several factors may account for these differences including the amount of sperm acquired at mating which might have been higher in Webb (2009) because those females were collected in an area closed to fishing. Females in the current study were also collected later in the reproductive cycle and spermathecal load may have decreased with increasing storage time (Sainte-Marie 1993).

The proportion of females with fresh ejaculate in the spermatheca varied among locations in this study. While females completing the molt to maturity are obligated to mate in order to produce a fertilized egg clutch and sperm reserves are a function of operational sex ratio (Sainte-Marie et al. 2002), specific factors determining the frequency of mating of multiparous females are not well understood (see Sainte-Marie et al. 2008). Based on mating indicators or measurement of sperm reserves, several studies in Alaska have inferred that most (~90 %) of multiparous female Tanner crab mate on an annual basis (Paul 1984, Webb 2009). The proportion of females with ejaculate remaining from recent mating varied among locations in this study with an overall proportion of 0.28 for SC3 and 0.42 for SC4 (Table 1.2). This suggests to us that the proportion of multiparous females identifiable as mated on an annual basis may be more variable than

previously recognized. In this study, increased proportions of females with fresh ejaculate were associated with increased mean sperm cell counts among locations for primiparous females and among location and shell condition groups for all females and may be useful as an indicator of relative differences in sperm reserves among areas (Table 1.3, Figure 1.5).

Comparison of quantitative indices of abundance or sex ratio from stock assessment surveys or models with empirical measures of variability in reproductive potential may assist fisheries managers in identifying levels of sex ratio or exploitation rate index which may result in female sperm reserves in excess of those needed for clutch fertilization. In this study decreasing sperm cell counts of SC3 females were associated with increasing exploitation rate index by location (Figure 5); for multiparous females the strongest associations were observed between mean spermathecal loads and survey or model estimates of sex ratio of large males to multiparous females. Interpretation of the robustness of these relationships is limited by samples sizes in some locations and years. However, it can be preliminarily concluded that increased exploitation rates may be inversely related to primiparous female sperm reserves and that differences in the amount of sperm accrued by multiparous females may vary as a function of the relative availability of legal-sized males during the mating season.

Defining sources of variability in female fecundity can provide understanding of how egg production may vary with biological characteristics or environmental factors. Previous studies have described seasonal variability in fecundity (Hilsinger 1976) and differences in the size-fecundity relationship between primiparous and multiparous female Tanner crab (Somerton and Meyers 1983). In addition to carapace width and shell condition, our results indicated that mean embryo weight is negatively associated with fecundity for Tanner crab in southeastern Alaska. Variability in mean embryo weight among female brachyuran crab has been described as a maternal effect and may be associated with variability in embryo and larval quality (Gimenez and Anger 2003). A similar relationship was also observed for *C. bairdi* in previous studies (Paul and Fuji 1989) and *C. opilio* from the Gulf of St. Lawrence, Canada (Sainte-Marie 1993). Similar to Somerton and Meyers' (1983) finding, lower fecundity, but of a greater magnitude (48% versus 30%), was observed between SC3 and SC4 females in this study. Differences in these estimates may be due to the separation of multiparous females into two shell condition classes in this study versus one in Somerton and Meyers (1983). In this study, fecundity of

multiparous (SC5) females was intermediate between SC3 and SC4 females (Table 1.5) and pooling of SC4 and SC5 females would have reduced the magnitude of differences in the size-fecundity relationship between SC3 and SC4 females. The fecundities of female Tanner crab in this study were generally similar to those from the northern Gulf of Alaska (Hilsinger 1976), but detailed comparisons were not possible because Hilsinger (1976) did not separate size-fecundity relationships between primiparous and multiparous females.

It is unlikely that sperm limitation was a substantial limiting factor in female egg production in this study. Mean prevalence of non-viable embryos and egg cases was low in all areas ($\leq 4\%$). Qualitatively, greater prevalence was not associated with lower mean sperm reserves for SC3 females in areas such as Icy Strait and Stephens Passage (Table 1.2, Figure 1.5). In support of this observation, both primiparous and multiparous females with zero sperm cells detected in the right spermathecae had fecundities either greater than or similar to those predicted by the ANCOVA model (Figure 1.6).

Our results suggest that fishery managers wishing to evaluate appropriate harvest levels among exploited stocks should concurrently assess female sperm reserves and viable embryo production on spatiotemporal scales relevant to management and compare trends with survey or model indices of the relative abundance of mature males and females. We observed that the sperm reserves of female Tanner crab in southeastern Alaska varied with reproductive ontogeny. For most individuals, both primiparous and multiparous, a high proportion of the sperm available for clutch fertilization was associated with recent mating, which was indicated by the presence of fresh, white, ejaculate in the spermatheca. The proportion of females with fresh ejaculate in the spermatheca by location was significantly correlated with variability in mean sperm cell counts and may be useful as an index of spatiotemporal variability in female sperm reserves. Further, decreased primiparous sperm reserves were significantly correlated with increased exploitation rate index. Differences in multiparous female spermathecal loads were associated with model and survey derived indices of sex ratio. If these relationships prove robust with additional years of data collection, it may be possible to define target or limit reference points for management based on functional relationships between these indices and female sperm reserves which could result in low risk of decreased viable egg production due to sperm limitation for female Tanner crab.

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Table 1.1. Size and sex categories for male and female Tanner crab used to calculate indices of sex ratio among locations in southeast Alaska.

Population Segment	Metric	Shell Condition	Carapace Width (mm)
Primiparous Female (PF)	Pot Survey CPUE	3	n/a
Multiparous Female (MF)	Pot Survey CPUE	4	n/a
All Mature Male (AMM)	Pot Survey CPUE	3-5	>109.3
Newshell Legal Male (NLM)	Pot Survey CPUE	3	>137.9
Oldshell Legal Male (OLM)	Pot Survey CPUE	4-5	>137.9
Legal Male Biomass (LMB)	Model Biomass Estimate	3-5	>137.9
Mature Male Biomass (MMB)	Model Biomass Estimate	3-5	>109.3

Table 1.2. Female sperm reserves and indicators of recent mating for Tanner crab by stock and for all stocks combined in Southeast Alaska in 2007. Mean spermathecal load (SL in g) and sperm cell count (SSC) of the right spermatheca and the proportion of females with fresh ejaculate (PFFE) and grasping marks (PFGM) for primiparous (SC3), multiparous (SC4), and multiparous (SC5) female Tanner. Significant differences (Tukey HSD, $p < 0.05$) in spermathecal load and sperm cell counts by shell condition and location are indicated by dissimilar letters. Means for all locations combined are presented for comparison.

Location	SC	N	Mean of SL (g)	SE of SL	Mean of SCC	SE of SCC	PFFE	PFGM
Glacier Bay	3	21	0.049 f	0.017	1.64×10^7 bc	5.79×10^6	0.29	
	4	9	0.134 abcd	0.023	1.09×10^7 abc	4.54×10^6	0.67	0.00
Holkham Bay	3	12	0.043 def	0.010	3.05×10^7 a	7.90×10^6	0.92	
	4	9	0.277 a	0.025	2.61×10^7 abc	8.07×10^6	1.00	0.44
	5	6	0.180 ab	0.030	9.83×10^6 abc	5.52×10^6	0.33	0.17
Icy Strait	3	5	0.024 f	0.022	5.94×10^6 c	5.39×10^6	0.20	
	4	10	0.172 abc	0.031	2.52×10^7 abc	7.66×10^6	0.60	0.20
Port Camden	3	8	0.045 cdef	0.016	3.01×10^7 abc	1.55×10^7	0.88	
	4*	3	0.177	0.025	3.21×10^7	3.04×10^7	1.00	0.33
Stephens Passage	3	11	0.031 ef	0.013	1.36×10^7 abc	4.54×10^6	0.09	
	4	12	0.305 a	0.031	2.16×10^7 ab	4.46×10^6	0.50	0.33
	5*	1	0.309		3.05×10^7		0.00	0.00
Thomas Bay	3	15	0.027 ef	0.005	2.01×10^7 abc	6.38×10^6	0.29	
	4	10	0.093 bcde	0.011	2.86×10^7 abc	1.18×10^7	0.80	1.00
	5	5	0.172 abc	0.026	1.96×10^7 abc	7.69E+10^6	0.60	0.40
All Locations	3	74	0.039	0.006	1.98×10^7	3.14×10^6	0.43	
	4	53	0.200	0.016	2.32×10^7	3.52×10^6	0.72	0.40
	5	12	0.187	0.021	1.56×10^7	7.69E+10^6	0.42	0.25

*Excluded from analysis due to limited sample size

Table 1.3. Sperm reserves with presence or absence of indicators of recent mating, fresh ejaculate or grasping marks, by shell condition group. Sample size, mean sperm cell count, and mean spermathecal load of the right spermatheca by shell condition for female Tanner crab collected in Southeast Alaska in 2007. *P*-values are reported from Mann-Whitney rank-sign tests of significant differences in mean sperm cell count and mean spermathecal load between females. Statistical comparisons and standard errors are not presented for SC5 females without grasping marks due to low sample size.

			Sperm Cell Count		Spermathecal Load	
Shell Condition	Fresh Ejaculate Present	N	Mean ± SE	p	Mean ± SE	p
3	Yes	42	$3.65 \times 10^7 \pm 5.80 \times 10^6$	<0.0001*	0.063 ± 0.008	<0.0001*
	No	31	$7.99 \times 10^6 \pm 2.03 \times 10^6$		0.021 ± 0.007	
4	Yes	38	$2.87 \times 10^7 \pm 4.51 \times 10^6$	0.0086*	0.21 ± 0.017	0.2142
	No	15	$8.97 \times 10^6 \pm 2.56 \times 10^6$		0.17 ± 0.033	
5	Yes	5	$2.66 \times 10^7 \pm 6.58 \times 10^6$	0.0424	0.21 ± 0.027	0.4649
	No	7	$4.82 \times 10^6 \pm 2.56 \times 10^6$		0.15 ± 0.029	
Shell Condition	Grasping Marks Present	N	Mean ± SE	p	Mean ± SE	p
4	Yes	32	$3.26 \times 10^7 \pm 6.81 \times 10^6$	0.0278*	0.20 ± 0.027	0.5569
	No	21	$1.69 \times 10^7 \pm 3.42 \times 10^6$		0.20 ± 0.019	
5	Yes	3	3.52×10^7		0.17	
	No	9	$1.70 \times 10^7 \pm 3.43 \times 10^6$		0.18 ± 0.025	

* Significant differences in sperm cell counts and spermathecal load with presence/absence fresh ejaculate by shell condition were adjusted with Bonferroni correction for multiple comparisons ($p < 0.017$), $p<0.05$ for sperm cell counts and spermathecal load with the presence/absence of grasping marks.

Table 1.4. Correlation between indicators of recent mating and sperm reserves among stocks of Tanner crab in Southeast Alaska in 2007. Pearson's correlation coefficient (r) and *p*-value of relationships among mean sperm cell count (SCC), mean spermathecal load (SL), and the proportion of females by area with fresh ejaculate (PFFE) or fresh grasping marks (PFGM) for primiparous (SC3) and oldshell multiparous (SC4) females. Significant ($p < 0.05$) relationships are indicated by boxes and shown in Figure 1.4.

	All Mature Females (SC3-5)			
	Mean SL		Mean SCC	
	r	*p*-value	r	*p*-value
PFFE	0.32	0.28	0.78	0.002
PFGM (SC4-5)	-0.23	0.61	0.73	0.06
	Primiparous Females (SC3)			
PFFE	0.57	0.22	0.91	0.011
	Multiparous Females (SC4)			
PFFE	-0.04	0.93	0.36	0.54
PFGM	0.76	0.13	-0.26	0.67

Table 1.5. Sperm reserves by shell condition, sex ratio, and exploitation rate for Tanner crab in Southeast Alaska in 2007. Pearson's correlation coefficient (r) and *p*-value of relationships by area between female mean spermathecal load (SL in g) and mean sperm cell counts (SCC), survey/model indices of sex ratio (defined in Table 1), and model estimates of exploitation index. Significant correlations ($p<0.05$) are indicated in bold and boxed relationships are presented in Figure 5.

	Primiparous Females (SC3)			
	Mean SL		Mean SCC	
	r	*p*-value	r	*p*-value
NLM CPUE/ PF CPUE	-0.47	0.35	-0.50	0.31
OLM CPUE/ PF CPUE	0.16	0.76	-0.34	0.51
OSM CPUE/PF CPUE	-0.73	0.10	-0.54	0.26
AMM CPUE/PF CPUE	-0.27	0.60	-0.40	0.43
LMB/PF CPUE	0.07	0.89	-0.37	0.46
MMB/ PF CPUE	0.50	0.30	-0.24	0.63
Exploitation Rate	-0.90	0.01	-0.75	0.08
	Multiparous Females (SC4)			
NLM CPUE/ MF CPUE	0.67	0.21	-0.05	0.21
OLM CPUE/ MF CPUE	0.86	0.06	0.06	0.91
OSM CPUE/MF CPUE	0.10	0.81	0.71	0.18
AMM CPUE/MF CPUE	0.71	0.17	0.01	0.98
LMB/MF CPUE	0.86	0.06	-0.14	0.82
MMB/ MF CPUE	0.48	0.40	-0.10	0.86
Exploitation Rate	-0.34	0.57	0.53	0.34

Table 1.6. Analysis of covariance (ANCOVA) results for female size-fecundity by shell condition for female Tanner crab from southeast Alaska in 2007. Factors and interaction terms preservation method (PM) and location that were not significant ($p < 0.05$) in the full model were removed. Parameter estimates +/- SE and significance of the effect of carapace width (CW), shell condition (SC), and mean egg weight (MEW) on fecundity was estimated from the reduced model. ($Y = e^{a+b_1 lnCW+b_2 SC+b_3 MEW}$).

Reduced Model

Source	d.f.	Sum of Squares	F-Ratio	*p*-value
ln CW	1	5.94	315.4	< 0.0001
SC	2	7.45	138.0	< 0.0001
MEW	1	2.59	198.0	< 0.0001
Error	130	2.39		
Factors removed from reduced model				
Location	5	0.02	0.28	0.92
PM	1	0.024	1.28	0.26
PM*MEW	1	0.054	2.89	0.091

Y	a	b_1		b_2	b_3	r^2	N
Fecundity	-0.07 +/- 0.70	3.17 +/- 0.22	SC3	0	-13,600 +/- 1,160	0.86	135
			SC4	0.48 +/- 0.03*			
			SC5	0.30 +/- 0.05*			

* ($p < 0.05$), Tukey HSD

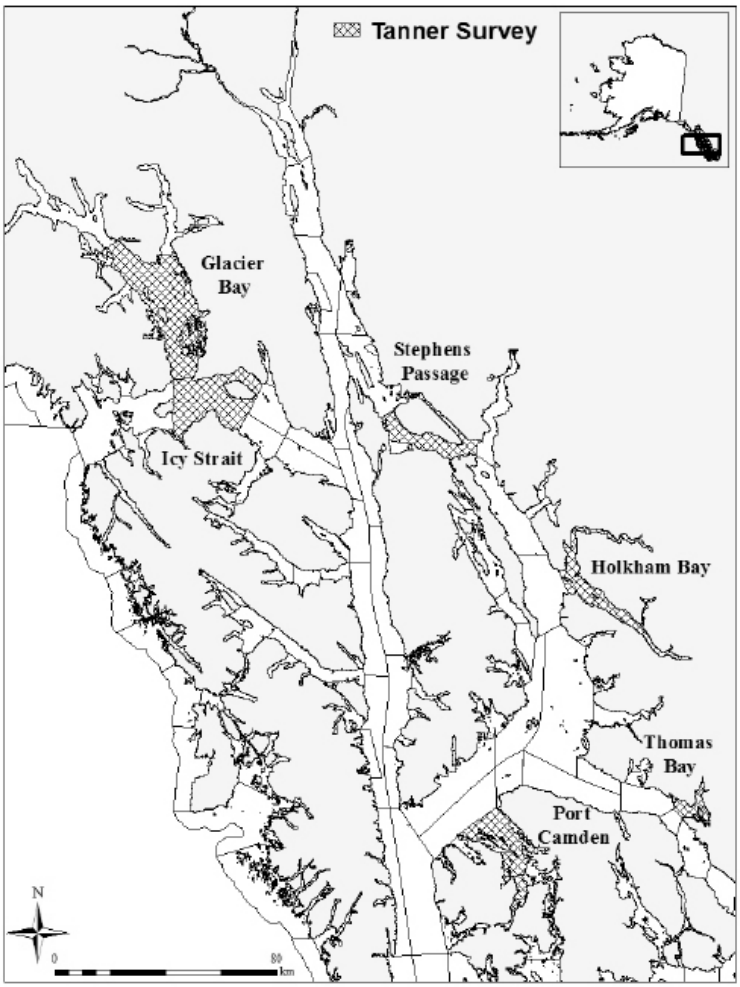

Figure 1.1. Map of Tanner crab stocks in Southeast Alaska (crosshatched) surveyed annually during the Alaska Department of Fish and Game Tanner crab stock assessment pot survey.

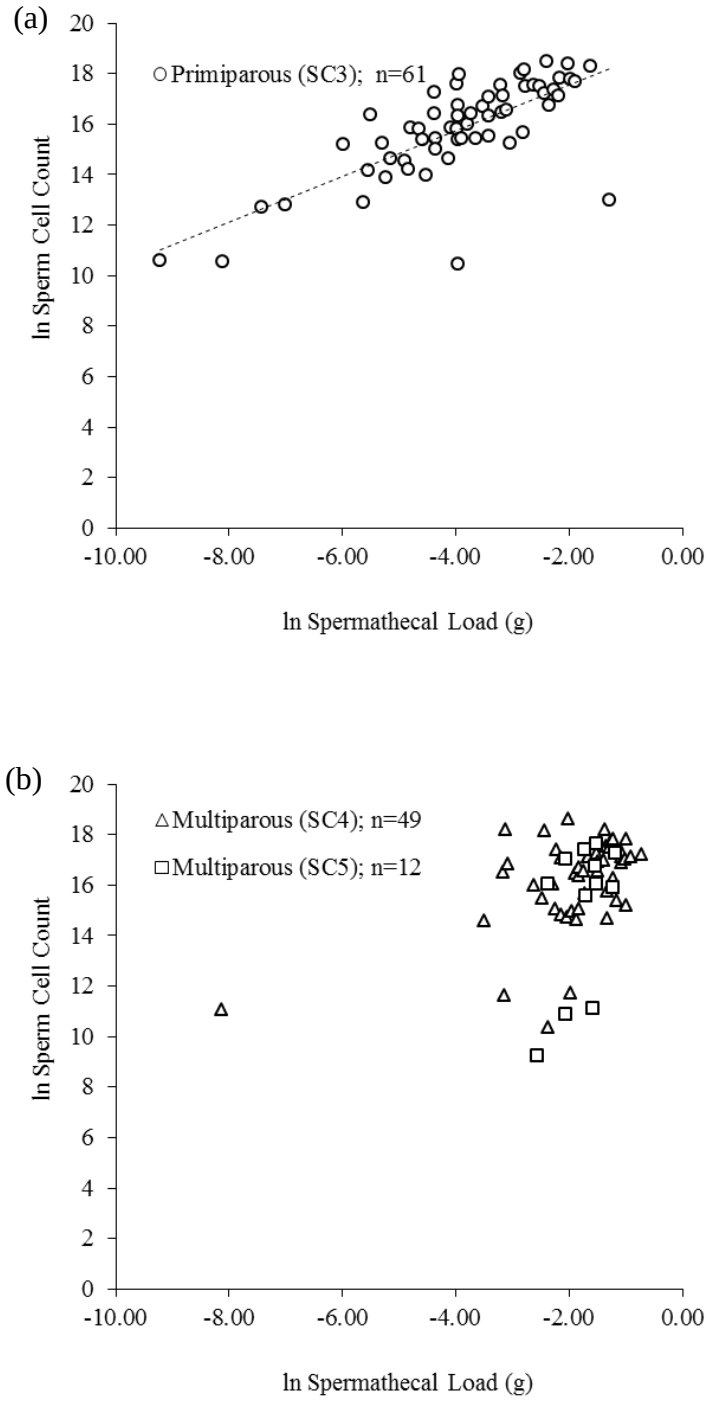

Figure 1.2. Female spermathecal load versus sperm cell count for (a) primiparous (SC3) and (b) old shell (SC4) and very old shell multiparous (SC5) female Tanner crab from southeast Alaska in 2007. Sperm cell counts increased significantly with spermathecal load for primiparous females ($\ln y = \ln 0.92x + 19.47$, $R^2 = 0.67$, $F = 119.7$, d.f. = 70, $p < 0.0001$), but were not linearly related for multiparous (SC4 or SC5) females. Eleven SC3 females with spermathecal loads or sperm cell counts estimated to be zero were excluded from this analysis.

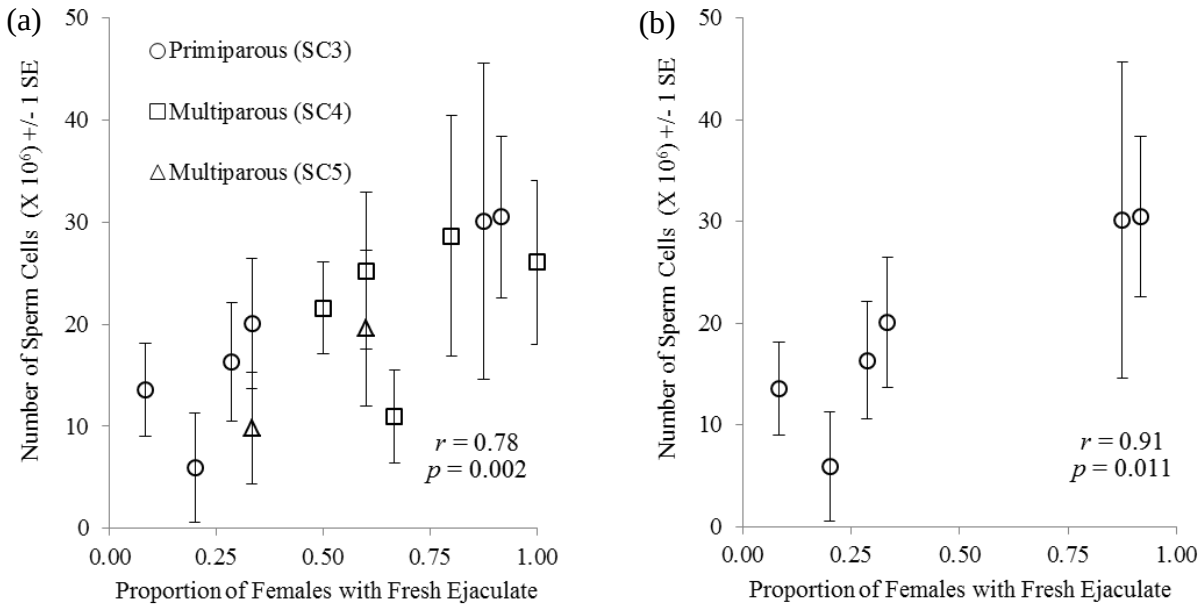

Figure 1.3. Association between fresh ejaculate and average sperm reserves for female Tanner crab in southeast Alaska in 2007 measured as the Pearson correlation (*r*) between mean sperm cell counts and the proportion of females with fresh ejaculate (PFFE) by location and shell condition. Correlations were significant ($p < 0.05$) for both comparisons. Data are also presented in Table 4.

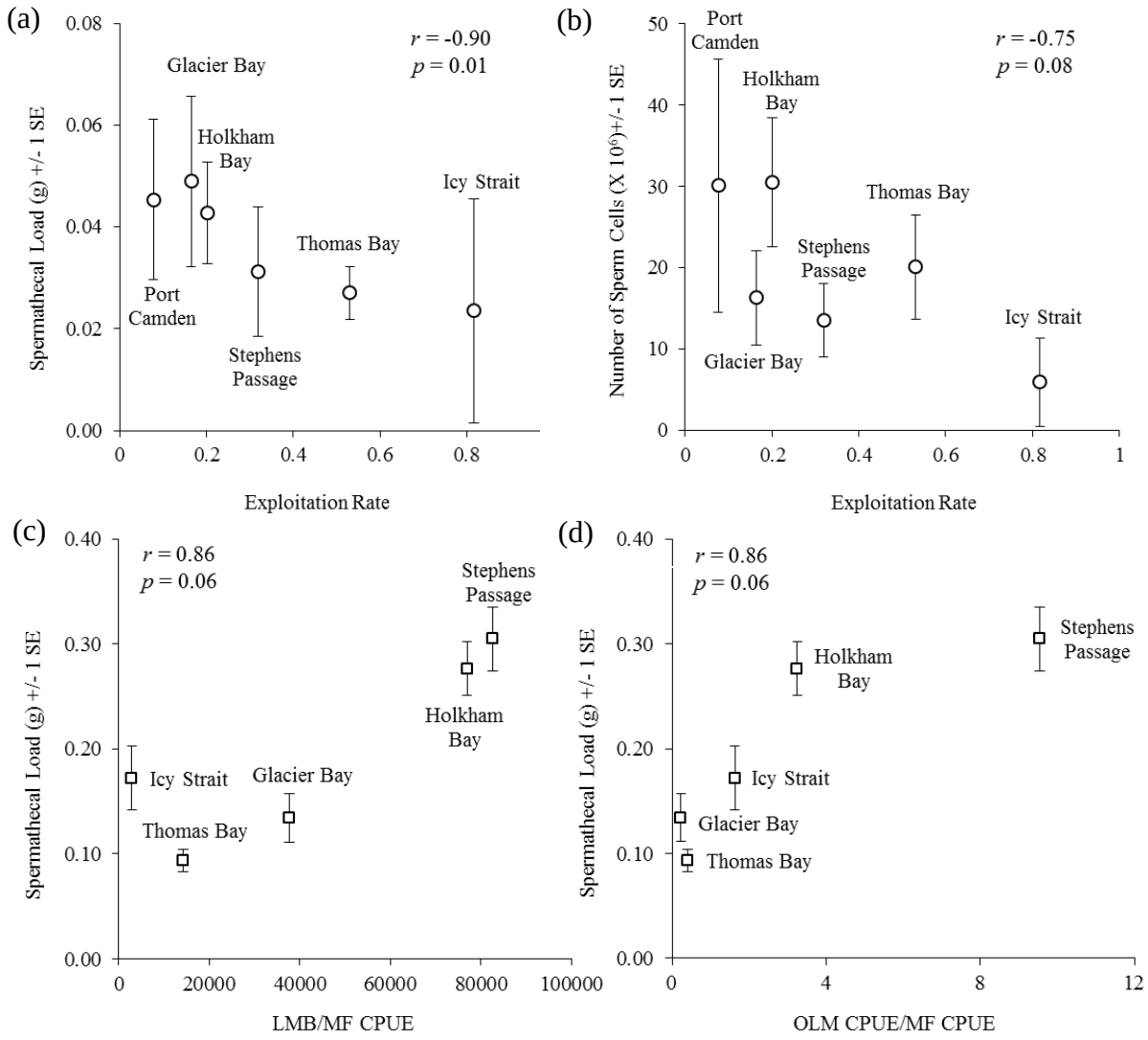

Figure 1.4. Association among exploitation rate, sex ratio, and sperm reserves for female Tanner crab in Southeast Alaska in 2007. Pearson's correlation coefficient (*r*) and *p*-value of relationships between (a) mean spermathecal load of primiparous (SC3) females and index of exploitation rate by area, (b) mean sperm cell counts of primiparous (SC3) females and index of exploitation rate by area, (c) mean spermathecal load of multiparous (SC4) females and the ratio of model estimated legal-size male biomass (LMB) to the survey CPUE of multiparous females (MF CPUE) by area, and (d) mean spermathecal load of multiparous (SC4) females and the ratio of survey CPUE of oldshell (SC4+), legal-size males (OLM) to the survey MF CPUE.

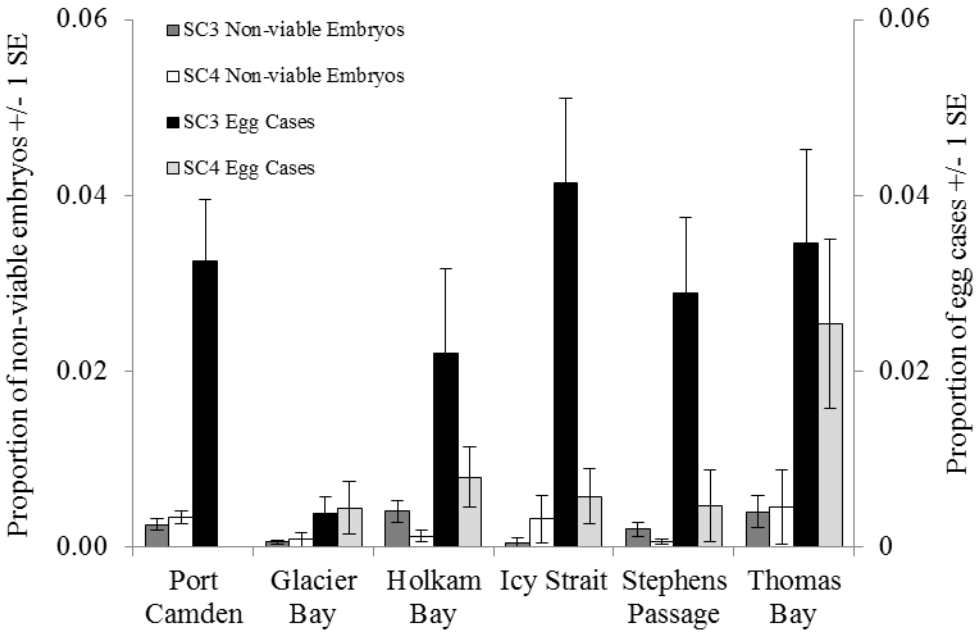

Figure 1.5. Variability in embryo viability for Tanner crab in Southeast Alaska, estimated as the proportion of egg cases to viable embryos and non-viable to viable embryos by location for female. Values were determined from counted subsamples (>400 embryos/female) for primiparous (SC3) and multiparous (SC4).

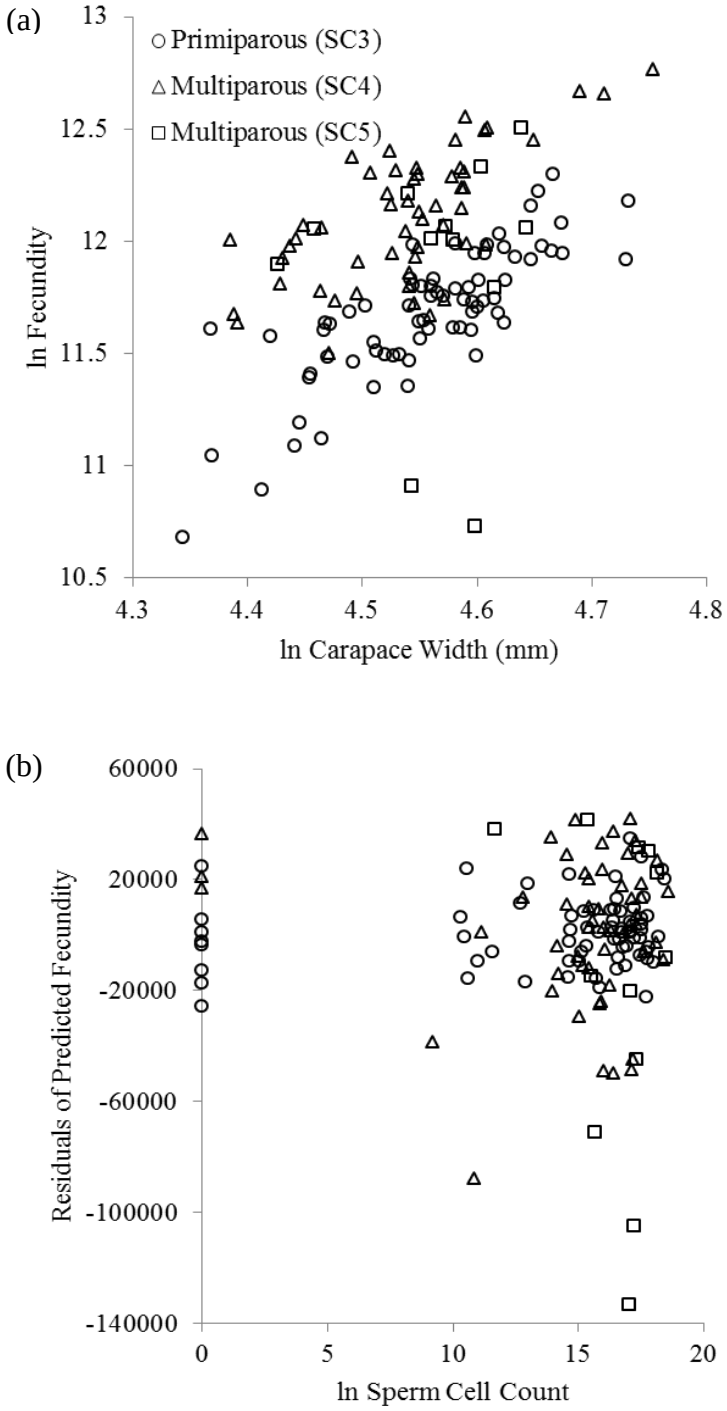

Figure 1.6. Female size-fecundity and sperm reserves-fecundity residuals versus fecundity for Tanner crab in Southeast Alaska in 2007. (a) The natural logarithm of female carapace width (mm) vs natural logarithm of fecundity and (b) the natural logarithm of sperm cell count of the right spermathecae versus the model standardized residuals of fecundity (prediction equation in Table 5) for primiparous (SC3), multiparous (SC4), and multiparous (SC5) female Tanner crab.

Chapter 2

Developing biological reference points for crustacean fisheries: the contribution of fecundity and embryo quality to reproductive potential of eastern Bering Sea snow crab[1]

Abstract

The incorporation of reproductive potential in the development of biological reference points is a pressing fishery management need for eastern Bering Sea (EBS) snow crab, *Chionoecetes opilio*. We collected female EBS snow crab from 2007 to 2009 to investigate seasonal and interannual variation in fecundity as a function of female size, shell condition (a proxy for relative age), and sperm reserves and in 2010 to examine biochemical aspects of embryo quality. Mean model-adjusted fecundity was highest for primiparous and young multiparous females and declined with advancing shell condition; patterns supported by variation in independent indices of clutch size. EBS snow crab had lower fecundity-at-size than conspecifics from Canada and Japan. Embryo carbon content (% C), a potential indicator of embryo quality, was similar between primiparous and multiparous females sampled in 2010, but was positively associated with higher than average fecundity. Indicators of low female sperm reserves or lack of recent mating were associated with decreased fecundity. Seasonal comparison of size-fecundity relationships suggested that embryo loss during brooding was minimal. Reductions in EBS snow crab reproductive potential may be associated with varying demographic structure, environmental factors and mating dynamics.

[1] Webb, J.B., Stichert, L.M., Eckert, G.L., and G.H. Kruse. Developing biological reference points for crustacean fisheries: the contribution of fecundity and embryo quality to reproductive potential of eastern Bering Sea snow crab. Prepared for submission to Canadian Journal of Fisheries and Aquatic Sciences.

2.1 Introduction

Information is needed to incorporate robust estimates of reproductive potential, as an index of population productivity, into fishery management for eastern Bering Sea (EBS) snow crab, *Chionoecetes opilio*. Landings and ex-vessel value of the EBS fishery peaked in the 1990s, declined dramatically, and subsequently continued at low levels (Figure 1). Current EBS snow crab fishery management uses the biomass of mature males as the only index of stock reproductive potential (Turnock and Rugolo 2011), thus development of improved indices based on female reproductive potential is a pressing need. Female reproductive output provides the initial input (egg production) from which density-dependent and density-independent processes ultimately regulate cohort strength (Caputi 1993, Lipcius and Stockhausen 2002, Houde 2008). Biomass-based indices of reproductive potential assume that stock egg production is proportional to biomass (Rothschild and Fogarty 1989). However, this relationship may diverge for species in which fecundity or embryo quality vary strongly with maternal factors (Lambert 2008), with subsequent impacts on estimation of biological reference points and perceptions of stock resiliency to fishing (Murawski 2001, Marshall et al. 2006). Incorporation of detailed information on reproduction (e.g. maturity, sex ratio, and egg production) can improve estimation of relationships between reproductive potential and recruitment particularly for species with highly variable reproductive traits (Morgan et al. 2011).

Among decapod crustaceans, fecundity and embryo quality can vary with maternal characteristics and environmental conditions. Patterns of increasing fecundity with increasing maternal size are generally conservative (Hines 1982, Reid and Corey 1991), and egg clutch size is constrained allometrically by both the volume of the body cavity and variation in egg size (Hines 1982). Fecundity-at-size can vary substantially among stocks and years for commercially important crabs (Haynes et al. 1976, Prager et al. 1990, Swiney et al. 2010). Factors potentially influencing size-specific fecundity include maternal age relative to maturity (Sainte-Marie 1993), variation in maternal condition through bottom-up processes (Wells 2009), density-dependence (Prager et al. 1990), variation in the physical environment (Hannah et al. 1995), brood sequence in species with multiple annual broods (Darnell et al. 2009, Verisimo et al. 2011), mating dynamics/fertilization success (Rondeau and Sainte-Marie 2001, Sainte-Marie et al. 2002), and embryo loss during brooding (Kuris 1990). Maternal size and reproductive history (Gimenez and Anger 2001, Ouellet and Plante 2004, Verisimo et al. 2011), embryo incubation temperature

(Amsler and George 1984, Fischer et al. 2009), and salinity during embryo incubation (Gimenez and Anger 2001, Bas et al. 2007) can influence embryo quality (size, energy content, and biochemical composition) with subsequent impacts on larval developmental rate, size, lipid content, and survival under starvation conditions (Racotta et al. 2003, Gimenez 2006).

Factors known to influence the size-fecundity relationship for snow crab include maternal size, maternal age relative to maturity, embryo loss, and oviposition of unfertilized eggs due to inadequate sperm availability (sperm limitation). Female snow crab have a terminal (final lifetime) molt to maturity (Watson 1970). Age relative to terminal molt is indexed by assignment of a subjective shell condition index (SC) based on the accumulation of epibionts and wear on the exoskeleton (Jadamec et al. 1999, Ernst et al. 2005). On an annual reproductive cycle, primiparous females (carrying the first egg clutch of ontogeny) are SC2 (new shell), whereas young, multiparous females bearing the second or third clutch of ontogeny are SC3 (old shell), and old, multiparous females carrying the fourth or greater clutch are generally classified as SC4 (very old shell) or SC5 (very, very old shell) (Jadamec et al. 1999, Ernst et al. 2005). Snow crab fecundity increases with female size (Haynes et al. 1976), and primiparous females have reduced fecundity-at-size compared to young, multiparous females (Sainte-Marie 1993). Among EBS females variability in mean clutch size index increases with increasing shell condition index, and mean clutch size index is lower for SC4 and SC5 than younger females (Orensanz et al. 2005). Reductions in fecundity with advanced shell condition in Japan were attributed to senescence with associated reductions in energy allocation to reproduction (Kon and Adachi 2006, Kon et al. 2010). Decreased fecundity of primiparous females in the Gulf of Saint Lawrence (eastern Canada) was attributed to egg loss during post-oviposition mating at male-biased sex ratios and sperm limitation at female-biased sex ratios (Sainte-Marie et al. 2002), and primiparous females with very low sperm reserves extruded unfertilized egg clutches in the laboratory (Rondeau and Sainte-Marie 2001). Estimates of embryo loss during brooding are highly variable and range from no detectable difference to a 50% decrease, with greater decreases for larger females (Kon 1974, Sainte-Marie 1993, Comeau et al. 1999). Biennial reproduction results in a protracted ~2 y duration of embryo development for female snow crab at temperatures less than approximately 1° C (Moriyasu and Lanteigne 1998, Sainte-Marie et al. 2008). Primiparous females, reproducing biennially, would be observed with egg clutches in the intermediate stages of development (Moriyasu and Lanteigne 1998) or with the second clutch of ontogeny as SC3

(Ernst et al. 2005). Potential interactions between varying reproductive tempo (annual/biennial) and the onset of reduced fecundity due to senescence have not been described.

The objective of this study was to improve the assessment of reproductive potential for female EBS snow crab by investigating variability in fecundity and embryo quality. Variability in size-fecundity was evaluated by year, season, and maternal characteristics (size, shell condition, and indicators of varying sperm reserves) for three years (2007 to 2009) and relationships between embryo size/elemental composition and maternal characteristics were investigated in 2010.

2.2 Methods and Materials

2.2.1 Specimen collection

Mature female snow crab, identified by a disproportionately large abdomen relative to immature females, were sampled in June and July of 2007-2009 during annual bottom trawl surveys conducted by the US NOAA National Marine Fisheries Service (NMFS, Chilton et al. 2011). Females of shell condition SC2, SC3, SC4 and SC5 (Jadamec et al. 1999) were sampled in proportion to the observed number of female snow crab of each shell condition group at each survey station. Sampled locations and sample size at each location varied annually, reflecting variability in female distribution (Figure 2). A total of 1,011 females (563 SC2, 276 SC3, 143 SC4, 29 SC5) were collected live, tagged, maintained live or frozen depending on condition, and shipped to laboratory facilities in Juneau or Kodiak, Alaska. Crab that died during collection or shipping were frozen whole until processing. Live females were maintained in large aquaria (>700 L) in the laboratory at temperatures similar to those *in situ* (0.0 to 2.0 °C) for periods of one to four weeks until processing. Since bottom trawl surveys occur only during the summer, ovigerous female snow crab were opportunistically collected from February to May of 2008 (N=102) and 2010 (N=66) as bycatch in groundfish fisheries by the North Pacific Groundfish Observer Program. Collection locations were constrained to the area fished by observed vessels in the southern and central portion of the snow crab distribution, and females were frozen aboard the fishing vessel and shipped to Juneau.

For evaluation of embryo quality, females (22 SC2, 25 SC3, and 17 SC4) with embryos in the early stages of development (gastrula or earlier, Moriyasu and Lanteigne 1998) were sampled from the EBS in June 2010 (Figure 2) during the annual bottom trawl survey. Females were held

live in circulating seawater tanks aboard the survey vessel, and shipped via airfreight to the University of Alaska Fairbanks laboratory in Juneau, Alaska, where they were held in 700 L aquaria with flow-through, circulating seawater chilled to 1.5 - 2.0° C.

2.2.2 Clutch fullness index

Weighted mean clutch fullness index (CFI) by shell condition group and year (1994-2010) were estimated from data collected during annual stock assessment surveys conducted by NMFS. CFI is a categorical assignment of egg clutch size based on a visual index of the volume extent of the egg clutch relative to the female abdomen with levels of 6 – full, 5 - 0.75, 4 - 0.5, 3 - 0.25, 2 - 0.125, 1 – empty or trace (Jadamec et al. 1999. Orensanz et al. 2005).

2.2.3 Laboratory data collection

Laboratory data collected from live females included carapace width (measured to the nearest 0.1 mm), SC, egg clutch color (Pantone™), grasping marks on legs, ovary color (Pantone™), ovary % fullness (25% increments relative to examples in Jadamec et al. 1999), ovary dry weight (1 in 3 sampling rate), egg viability based on the presence of cleavage and embryo structure, embryo development stage (Moriyasu and Lanteigne 1998), spermathecal fullness (25% intervals, where a 100% full spermathecae was defined as the absence of a visible empty portion of the spermathecae, and empty was defined as a lack of visible material in the spermathecae), presence of fresh ejaculate (Duluc et al. 2005), and CFI. The same data were collected from frozen specimens from survey and observer collections except when degraded condition of the ovary or embryos precluded reliable assessment of embryo viability or ovary fullness/weight. Fecundity was determined as the quotient of the mean embryo dry weight (determined from two replicate subsamples of >150 embryos) and dry weight of the egg clutch stripped from the pleopods. All embryos were dried at 60° C for >48 h in a drying oven and weighed to the nearest 0.1 mg with an Ohaus® DV215CD analytical microbalance.

Females collected in 2010 were processed as above with additional data collected on embryo volume and proximate biochemical composition. Mean embryo dry weight was estimated from six subsamples of ~50 embryos per sample and fecundity was calculated as the dry weight of the egg clutch divided by the mean embryo dry weight. Embryo volume was estimated by measurement of 10 live embryos per female photographed at 2.5x magnification on a Leica M80

stereomicroscope. The diameter of each embryo was estimated as the mean of its major and minor axis (Sainte-Marie 1993) measured using ImageJ image analysis software and embryo volume was calculated as a spheroid $V = \frac{4}{3}\pi 1/2\bar{d}^3$, where $\bar{d}$ was the mean of major and minor axes (Brante et al. 2003). Subsamples of the dried embryo clutches (~80 mg) of 16 SC2, 17 SC3 and 15 SC4 were randomly selected and analyzed for proximate biochemical composition by measurement of elemental composition of carbon, hydrogen, and nitrogen (CHN) by combustion methods (Gnaiger and Bitterlich 1984).

2.2.4 Statistical analysis

Data included in analyses of fecundity and embryo quality early in the reproductive cycle were screened to limit analysis to females with egg clutches in the early stages of development and without evidence of hybridization. Snow crab can hybridize with a congener the Tanner crab (*C. bairdi*). Tanner crab smaller embryos than snow crab (Haynes et al. 1976) as do morphologically identified hybrids (L. Slater, unpublished data). Females identified morphologically as snow crab, but with mean embryo weights similar to Tanner crab, indicating they were possibly *C. opilio* X *C. bairdi* hybrids, were excluded from comparisons using a threshold mean embryo weight of 4.80 X 10^{-5} g (J. Webb and L. Slater, unpublished data). Females in the intermediate year of biennial reproduction were excluded based on embryo development (post-gastrula) and ovary fullness (>50%). Final datasets included 453 SC2, 235 SC3, 136 SC4, and 29 SC5 (n=853) females for fecundity analyses and 22 SC2, 15 SC3, and 17 SC4 females for embryo quality. Potential differences in fecundity and clutch dry weight as a function of female carapace width, mean embryo dry weight, shell condition, year of collection, and location (random covariate) were evaluated using linear mixed models for females collected during the summer survey from 2007 to 2009. Variability in mean embryo dry weight was evaluated by the same approach, as a function of female size, shell-condition, and year of collection. Model-adjusted means of the dependent variables are reported throughout. Quantile regression (Koenker 2006) by shell condition group at the 5^{th} and 95^{th} percentiles (proxies for minimum and maximum fecundity at size) were used to evaluate possible changes in fecundity-at-size between shell condition groups with years pooled. ANOVA was used to evaluate differences in standardized fecundity residuals from the linear mixed model by shell condition group for females with or without fresh ejaculate in the spermathecae and among spermathecal

fullness classes for SC2 and SC3 females. SC4 and SC5 females were excluded from comparisons of fecundity residuals among spermathecal fullness classes due to low sample sizes. Differences in size-fecundity relationships between summer (early in the reproductive cycle) and spring (late in the reproductive cycle) collections by fishery observers were evaluated by ANCOVA using only females collected south of 58 ° N in the summers of 2007 and 2009, because spring collections were confined to the southern portion of the snow crab distribution. The fixed effect year was defined as the year of embryo extrusion for this analysis to facilitate comparisons (i.e. females collected by fishery observers late in embryo development in the spring were grouped with females collected the summer prior). A linear mixed model with carapace width and CFI as predictors was also used to estimate the slopes and intercepts and their standard errors of the size-fecundity relationship by CFI group.

Relationships among mean embryo volume, mean embryo dry weight, elemental composition (% C, % H, % N, and C/N), and elemental weight per embryo (C, H, and N in μg/embryo), maternal carapace width, maternal shell condition, and fecundity of females collected in 2010 were evaluated by analysis of covariance (ANCOVA). Female carapace width, shell condition, and their interaction were treated as fixed effects. Mean embryo dry weight, elemental composition (% C, % H, % N, and C/N), and elemental weight per embryo were modeled as dependent variables. Associations among embryo characteristics (volume and weight), elemental composition (% C, % H, and % N), and model-adjusted fecundity residuals were evaluated with estimates of pairwise Pearson correlation (*r*).

Mean female CFI by shell condition and year was estimated from the NMFS survey database as the mean CFI among all surveyed stations weighted by the mean density of female snow crab for each combination of shell condition and clutch fullness index within each year. Differences in the weighted mean CFI and the coefficient of variation of CFI among shell condition groups were tested with a Kruskal-Wallis test and Dunn's post-hoc test (Zar 1999).

A top-down procedure was used for mixed-effects model selection (Zuur et al. 2009). Normality of model residuals was evaluated by normal-quantile plots and the Shapiro-Wilk test. Homogeneity of variance was evaluated by plots of model residuals versus predicted values. When necessary, square root or natural logarithm transformations were applied to the dependent

variable to achieve homogeneity of variance of the residuals. Influential outliers were identified by Cook's distance, and model robustness to influential outliers was evaluated by fitting models both including and excluding outliers. The threshold significance level for all statistical analyses was $\alpha=0.05$. Post-hoc Tukey HSD tests of the model-adjusted mean were conducted for significant results with more than two comparisons. Quantile regression was conducted using the R package quantreg in R 2.15.0 (R Development Core Team 2012) with standard errors of the slopes and intercepts estimated by bootstrap. All linear model and CFI analyses were conducted in R 2.15.0 or JMP 9.02 (SAS Institute, Cary, NC).

2.3 Results

2.3.1 Size-fecundity relationships among shell condition classes and years

Fecundity was positively associated with female size and varied among shell condition groups and years (Table 2.1, Figure 2.3A). SC3 females had the highest overall fecundity but this pattern varied by year. This group had lower fecundity in 2008 compared to 2007 and 2009, which resulted in significantly higher fecundity for SC3 compared to primiparous (SC2) females in 2007 and 2009 but not in 2008 (Table 1, Figure 3A). SC4 and SC5 females generally had lower fecundity than SC2 and SC3 females, although in some years low sample sizes resulted in high variance and low statistical power to detect differences (Table 1, Figure 3A). Fecundity of SC5 females was ~50% of the fecundity of SC3 females in 2007 and 2008, but this comparison was limited in 2009 owing to a small sample size (n=3) for SC5 females (Table 1, Figure 3A). Collection location, a random factor in the analysis, contributed ~21% of the residual variance to the size-fecundity relationship (Table 1).

The spread in fecundity-at-size varied with shell condition with greater variance in fecundity-at-size for SC3 and SC4 than SC2 females (Table 2, Figure 4). Quantile regression at the 95^{th} percentile showed increased maximum fecundity-at-size from SC2 to SC3 and decreased maximum fecundity at size for shell conditions SC4 and SC5 (Table 2, Figure 4). The relationship between size and fecundity degenerated for older animals, as fecundity did not increase with increasing carapace width for SC5 females (Table 2, Figure 4).

2.3.2 Mean embryo and clutch dry weight

Mean embryo dry weight differed significantly among years and shell condition groups but not with maternal size (Table 2.3, Figure 2.3B). Mean embryo dry weight was similar across shell condition classes in 2007, was lower for SC2 than for SC3, SC4 or SC5 in 2008, and increased with increasing shell condition in 2009 (Table 2.3, Figure 2.3B). Among all females in this study, mean (± SE) embryo dry weight was 66.92 ± 4.43 µg. Clutch dry weight differed significantly among years and shell condition groups (Table 2.4, Figure 2.3C). Carapace width and shell condition were the strongest predictors of clutch dry weight, followed by year and mean embryo dry weight (Table 2.4). Egg clutch dry weight was highest for SC2 to SC4 females compared to SC5 females in 2007, higher for SC2 and SC3 females compared to SC4 and SC5 females in 2008, and did not vary across shell conditions in 2009 (Table 2.4, Figure 2.3C). Mean (± SE) clutch dry weight of all females was 2.10 ± 0.04 g.

2.3.3 Sperm reserves and fecundity

Indicators of greater or more recent sperm reserves, measured as % spermathecal fullness and presence of fresh ejaculate, respectively, were positively associated with fecundity (Figure 5). Spermathecal fullness and the presence of fresh ejaculate were positively related among all females, as the proportion of females classified with fresh ejaculate increased with increasing spermathecal fullness from 13% in the 0-25% fullness category to 94% at 75-100% fullness. Model standardized fecundity residuals pooled by year were higher for SC3 than SC2 females (ANOVA, $F=5.02$, $p=0.002$) and for SC3 females with spermathecae 25%-50%, 51-75% or 76 - 100% full than with spermathecae 0-25% full (ANOVA, $F = 5.93$, $p<0.001$; Tukey HSD $p<0.05$; Figure 5). Model-standardized fecundity residuals did not vary significantly with spermathecal fullness for SC2 females. Model standardized fecundity residuals also varied among shell condition groups with the presence or absence of fresh ejaculate (ANOVA; $F_{7,830} = 4.94$, $p<0.0001$). The presence of fresh ejaculate was associated with a significant (Tukey HSD, $p<0.05$) increase in residual fecundity, a difference in residuals of 3,000-4,000 embryos, for SC3 and SC4 females but not for SC2 or SC5 females (Figure 5).

2.3.4 Embryo quality

Embryo quality, determined by embryo volume, dry weight, and elemental composition did not vary as a function of female size, but some differences were observed among shell condition

groups (Tables 5 & 6). Mean embryo dry weight, C weight per embryo, and H weight per embryo were significantly lower for SC4 than SC2 females, but a similar trend was not detected in embryo volume. Significant, positive, but generally weak (r = 0.3-0.4) correlations were observed among embryo volume, weight and elemental composition (Table 7, Figure 6). Mean embryo volume was positively correlated with mean embryo dry weight (Figure 6C). Embryo % C was positively correlated with embryo % N (Figure 6D) and fecundity residuals (Figure 6B).

2.3.5 Seasonal comparisons of size-fecundity relationships and reproductive status

Fecundity was similar among SC3 and SC4 females in both summer and spring, but very oldshell (SC4) females had significantly lower fecundity than SC3 females in both the summer and the spring (Table 8, Figure 7). Most (90%) females collected in the spring time were brooding clutches with eyed embryos, and 19% of females had egg clutches with evidence of hatching (egg cases mixed with eyed eggs) at the time of collection. Evidence of hatching was observed from March to June but not in February. Most (97%) females collected by fishery observers were SC3 or SC4.

2.3.6 Clutch fullness index

Eighty percent of the variation in fecundity was explained by female size and CFI (Table 10, Figure 8A). Weighted mean CFI pooled among years (1994-2010) varied significantly by shell condition group, with greater mean CFI for SC2 and SC3 females than SC4 and SC5 females (Kruskal Wallis; $\chi^2 = 28.9$, df = 3/68; $p<0.001$; Dunn $p<0.05$; Table 10). The coefficient of variation of CFI differed also among shell condition groups with a significantly higher CV for SC4 and SC5 than for SC2 or SC3 females (Kruskal-Wallis; $\chi^2 = 44.9$, df = 3/68; $p<0.001$; Dunn $p<0.05$; Table 10).

2.4 Discussion

Fecundity of sampled EBS snow crab varied among years and as a function of female size, shell condition, and sperm reserves and was well approximated by CFI, which could serve as a useful index of reproductive potential for fishery management. Comparison of size-fecundity relationships by shell condition group suggested that average fecundity was generally highest for both primiparous (SC2) and young, multiparous (SC3) females, and decreased thereafter with increasing female age. Estimation of variability in maximum and minimum fecundity-at-size by

quantile regression provided finer scale resolution and demonstrated 26% greater maximum fecundity at size of SC3 versus SC2 females. In the Gulf of Saint Lawrence and Japan young multiparous snow crab had 17-30% greater mean fecundity than did primiparous females (Sainte-Marie 1993, Kon and Adachi 2006), although not consistently across years (Sainte-Marie et al. 2008). The lower maximum fecundity of SC2 compared to SC3 females is likely attributable to limited body volume available for ovary maturation and energy allocation to both growth and reproduction prior to the molt to maturity (Sainte-Marie 1993), as in the congener, Tanner crab (*C. bairdi*, Somerton and Meyers 1983). The decreasing trend in both maximum and mean fecundity-at-size from SC3 to SC5 is likely a result of female senescence. The size-fecundity relationship of primiparous females was relatively stable through the relatively short duration of this study, a pattern that was also reflected since 2003 in the CFI time series. Variability in mean CFI of SC2 females was greater prior to 2003 and may indicate the influence of other factors, such as mating success, on CFI (e.g. Sainte-Marie et al. 2008). Multiparous female fecundity varied among years in response to differences in embryo size/weight, sperm limitation, and senescence. Pooled mean embryo dry weight of multiparous females (SC3, SC4, and SC5) in 2008 was ~22 % greater than in 2007, while clutch dry weight was similar or decreased, indicating a potential change in per embryo energy allocation. Per embryo energy allocation may be affected by maternal condition/nutrition or environmental factors during oocyte formation and maturation, but these patterns have not been comprehensively studied for female snow crab (Sainte-Marie et al. 2008). Variability in fecundity related to differences in mean embryo weight is unlikely to be detected by the current CFI monitoring protocol. Annual monitoring of the fecundity of SC2 and SC3 female snow crab has continued since 2009 in the EBS and will likely provide further insight into interannual variably mean embryo weight between these two groups.

Indicators of low sperm reserves were associated with reduced fecundity of multiparous females in this study. Lower mean residual fecundity, equivalent to ~ 10% reduction at mean female size, was observed for SC3 females with low spermathecal fullness and with an absence of fresh ejaculate for SC3 and SC4 females (Figure 5). Fresh ejaculate is an indicator of recent mating (Duluc et al. 2005) and increased sperm reserves (Webb and Bednarski 2010). Sperm limitation may occur for multiparous snow crab if sperm reserves stored from previous mating are absent or low and females are not mated prior to oviposition of the subsequent clutch (Rondeau and

Sainte-Marie 2001, Sainte-Marie et al. 2002, Slater et al. 2010). Sperm viability also decreases with extended duration of storage (Sainte-Marie et al. 2008). Sperm reserves of primiparous female EBS snow crab are lower than those typically observed for conspecifics from eastern Canada, with a high proportion classified with empty spermathecae by visual indicators (Figure 5) and quantitative assessment (Slater et al. 2010). There was no evidence of retention of mature oocytes in the ovary or high proportions of abnormally developing (e.g. unfertilized) embryos in the egg clutches of females with low residual fecundity and low spermathecal fullness (J. Webb and L. Slater, unpublished data). Association between lower fecundity and reduced sperm reserves could be a result of sloughing of unfertilized embryos due to sperm limitation or embryo loss due to post-oviposition mating (Rondeau and Sainte-Marie 2001, Sainte-Marie et al. 2002). Operational sex ratio and the maturity status (i.e. adolescent versus adult) of males present at the time of mating likely differ between primipara and multipara in the EBS (Somerton 1981, Armstrong et al. 2008) and further evaluation of relationships between these factors and quantitative measures of female sperm reserves in the EBS (c.f. Slater et al. 2010) will be valuable to provide further insight into these patterns.

Patterns of variability in mean CFI among shell condition groups (Table 10) were similar to those observed for mean fecundity (Figure 3). Statistical models with only CFI and female size as predictors explained a comparable proportion of the variation in fecundity (R^2=0.80) as did models integrating a suite of biological variables and structured by the sampling period (R^2=0.81, Table 1, Table 9). Analysis of pooled CFI estimated from stock assessment survey data showed long-term variability in CFI that increased with increasing shell condition, which was very similar to our observations of increased variability in fecundity with increasing shell condition. Cyclic variation in CFI was attributed to interactions among fluctuating recruitment, female movement, and declining female fecundity during periods of increasing average female age (Orensanz et al. 2005, Ernst et al. 2012). These patterns were visible in peaks and troughs of the CFI relationship, with troughs in CFI by shell condition occurring at circa 5-6 yr intervals, as abundant cohorts advance in shell condition/age (Ernst et al. 2012).

The lack of differences in the size-fecundity relationship between samples collected in summer and the following spring implied that a reduction in fecundity did not occur during the brooding period in the EBS. In support of this conclusion, a ~13% difference in fecundity was observed

between SC3 and SC4 in both summer and spring, suggesting that differences in the size-fecundity relationship are established prior to oviposition or early in embryo development and persist through embryo incubation without substantial modification due to embryo loss or embryo mortality for females of both shell condition groups. This finding is consistent with that of Sainte-Marie (1993) and contrasts with estimates of reductions in fecundity of 20% to 50% during incubation in other snow crab stocks (Kon 1974, Comeau et al. 1999).

Our observation of empty egg cases mixed with pre-hatch stage embryos, indicative of larval release during the spring (March to May) was consistent with previous studies, in which peak snow crab larval abundance was observed in April (Incze et al. 1987) and springtime hatching was inferred from staging of ovarian maturation and embryo development (Rugolo et al. 2005). None of the females collected in the spring in our opportunistic samples were SC2, which could be due to sampling outside the geographic distribution of SC2 females (Zheng et al. 2001, Ernst et al. 2005) or perhaps the transition from SC2 to SC3 occurred before the spring sampling (Ernst et al. 2005).

Relative (%) elemental composition did not vary with shell condition group for female snow crab sampled for embryo quality, but differences in mean embryo dry weight and elemental weight per embryo were observed. Observations of stability in % elemental composition with significant variation in mean embryo dry weight (Table 7) implies that differences in mean embryo dry weight are likely to indicate real differences in embryo energy content. Values of % C (47-55), % H (7-10), and % N (8-10) estimated for early stage snow crab embryos were similar to those estimated for early embryo stages of caridian shrimps (Clarke 1993, Anger et al. 2002, Urzúa et al. 2012), and other brachyuran crabs (Fischer et al. 2009). The consistency of results across decapod crustacean taxa suggests that variation in embryo provisioning may be observed as differences in embryo dry weight versus variability in the elemental composition (Anger et al. 2002, Fischer et al. 2009). Embryo dry weight is a more reliable indicator of energy content than embryo diameter for embryos early in development (Clarke 1993, Jaeckle 1995, Moran and McAlister 2009) and C weight per embryo is considered a dependable index of energy content (Gnaiger and Bitterlich 1984, Urzúa et al. 2012). Lower mean embryo dry weight in SC4 versus SC2 females in the embryo quality sampling could be interpreted to indicate a trend toward reduced embryo quality with increasing age post-maturity (e.g. elapsed reproductive cycles).

However, this pattern was based on smaller samples at fewer locations (Figure 2) than the fecundity collections in prior years and the pattern of mean embryo dry weight by shell condition observed in the fecundity portion of our study in 2008 and 2009 (Figure 3B) contrasted with that observed in the embryo quality study (Table 6). Interestingly, fecundity residuals for females of all shell conditions collected in 2010 were positively correlated with % C (Figure 6C), suggesting a potential relationship between higher embryo energy content and higher than average fecundity for females regardless of shell condition. Linkages among environmental variability, maternal condition, and embryo size and energy content have been demonstrated for other brachyuran crabs (Brante et al. 2003, Gimenez and Anger 2001, Bas et al. 2007, Wells 2009), and these factors may also influence snow crab fecundity and embryo quality.

Comparison of embryo characteristics measured in this study with other snow crab stocks was limited due to the scarcity of published data. The mean embryo diameter of snow crab in this study (618 µm) was smaller than those previously observed for primiparous snow crab in the EBS (~660 µm), females of mixed reproductive history from Gulf of Saint Lawrence (650 µm), and primiparous females from the Chukchi Sea (710 µm) (Jewett 1981). The diameter of snow crab embryos increases with advancing development. For instance, embryo diameters of ~ 580 and 644 µm post-oviposition and ~830 and 772 µm eyed, prehatch were reported, respectively, by Sainte-Marie (1993) and Moriyasu and Lanteigne (1998). Embryos measured for this study were in the early stages of embryo development (~gastrula) and likely had been developing for several months (1-5 mo) at the time of collection (Moriyasu and Lanteigne 1998, Webb et al. 2007). Primiparous females in the Chukchi Sea (Jewett 1981) were sampled later in the year (autumn) and may have been in more advanced stages of embryo development (e.g. larger diameter) than those in our study.

The EBS snow crab stock is broadly distributed and spatially structured (Zheng et al. 2001, Ernst et al. 2005, Parada et al. 2010) with little overlap in the distribution of SC2 and SC3-SC5 females. Yet spatial factors were estimated to contribute only 21% of the residual variation (e.g. ~ 5% of total variation) in fecundity (Table 1). This may imply that the potential location-specific environmental and habitat influences on fecundity are captured as model fixed effects rather than being detected at specific locations. Interannual differences in mean embryo dry weight and fecundity by shell condition group and year may be associated with ontogenetic

habitat shifts, variability in the physical environment, and the associated influences on maternal condition and reproduction. Both male and female snow crab settle to the benthos and grow to maturity at depths of 50 – 100 m depth in the middle domain of the EBS (Zheng et al. 2001, Orensanz et al. 2004), a region interannually and seasonally dominated by cold bottom water, e.g., -1.5 to 4 ° C (Orensanz et al. 2007, Mueter and Litzow 2008). Following the molt to maturity, females undertake a cross shelf ontogenetic migration into the deeper, warmer (~ 2 to 5° C) waters of the outer domain in a primarily southeasterly direction (Orensanz et al. 2004, Ernst et al. 2005). In addition to differences in prevailing thermal conditions, primary and benthic productivity dynamics likely vary between these domains (Iverson et al. 1979) with potential influence on maternal condition and embryo characteristics. A prolonged duration of ovarian maturation (~3 y; Alunno-Bruscia and Sainte-Marie 1998) leads to the production of the primiparous egg clutch, while ovarian maturation takes place in one (annual) or two (biennial) years for multiparous females. Most multiparous females in the outer domain of the EBS are likely on an annual reproductive cycle (Armstrong et al. 2008). Associated with a shorter duration of ovarian maturation (~ 1 y) period, their oocytes and embryo characteristics (e.g. mean dry weight) may exhibit greater variability in relation to the influence of varying environmental or primary production dynamics on maternal condition and energy allocation. The spatiotemporal distribution of mating may also vary between primiparous and multiparous females with mating dispersed in space and time for primiparous females and aggregated in space and time for multiparous females (Ernst et al. 2005, see discussion in Sainte-Marie et al. 2008).

Qualitative comparison of best-fit size-fecundity relationships among snow crab stocks imply that both primiparous and multiparous EBS females have lower fecundity-at-size than conspecifics from Wakasa Bay, Japan, and the northwestern Gulf of Sainte-Lawrence, Canada (Figure 9). Estimated fecundity-at-size for primiparous females sampled in the EBS in 1968 (Haynes et al. 1976) and Chukchi Sea in 1976 (Jewett 1981) were comparable to that of primiparous females sampled in this study (Figure 9). Potential mechanisms underlying differences in the size-fecundity relationship among stocks have not been assessed for snow crab. However, the possibility of stock-specific differences in reproductive potential per unit of mature female biomass highlight the importance of studies to quantitatively evaluate reproductive

parameters and develop stock-specific understanding of the life history processes influencing stock productivity and persistence (Orensanz et al. 2004, Parada et al. 2010, Ernst et al. 2012, Burgos et al. 2013).

Patterns of size-fecundity by shell condition observed in this study agreed well with variability in independent long-term observations in CFI in the EBS and suggested that monitoring of CFI is a relatively effective method of appraising female reproductive potential. Improved understanding of life-stage specific (primipara and multipara) mating dynamics *in situ* and pre-oviposition factors influencing ovarian maturation and oocyte production are needed to clarify the relative importance of these processes in shaping female reproductive potential. Monitoring of female sperm reserves (e.g. Slater et al. 2010), egg fertilization, and quantitative evaluation of sperm reserves relationships among primipara and multipara similar to efforts for snow crab in the Gulf of Saint Lawrence, Canada (Sainte-Marie et al. 2008), may provide further insight into factors mediating the probability and magnitude of sperm limitation with female size and shell condition. Relative to spatiotemporally comprehensive collection of CFI data, studies of size-fecundity relationships may have limited ability to describe stock-level variability in reproductive potential for high-abundance; broadly-distributed crab stocks (e.g. Ernst et al. 2012). Nevertheless, such studies provide contextual insight into the factors influencing the size-fecundity relationship including variability in embryo characteristics, embryo viability, and sperm limitation. Our results suggest that variability in reproductive potential per unit biomass or abundance is lower for primipara than multipara. If spatiotemporally persistent, the patterns of variability in embryo dry weight and carbon content observed between SC2 and SC3/SC4 females in both the fecundity and embryo quality components of this study may bear further attention due to the potential for "carry-over" effects of embryo quality to the larval and juvenile stages. Biennial reproduction was not addressed in this study, but consideration of this factor will be a key aspect of future efforts to define biological reference points for fishery management based on interannual variability in female reproductive potential and relationships to the male-only fishery (e.g. sperm demand).

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Table 2.1. Results of linear mixed model analysis of variation in female size-fecundity with the effect of shell condition index (SC), year of collection (YEAR), carapace width (CW) nested within shell condition index and year, mean embryo dry weight (MEW) nested within shell condition index and year, and location of collection (LOC) estimated as a random component of the residual variance, on fecundity of eastern Bering Sea snow crab with embryos in the early stages of development from summer 2007 to summer 2009.

		N	R^2	RMSE
WHOLE MODEL FIT		853	0.80	3895
FIXED EFFECT TESTS		df	*F*	*p*
	CW[SC, YEAR]	12	89.1	<0.0001
	MEW[SC,YEAR]	12	19.5	<0.0001
	SC	3	15.1	<0.0001
	YEAR	2	3.77	0.044
	YEAR*SC	6	5.07	<0.0001
COMPONENTS OF RESIDUAL VARIANCE		Variance	SE	% of Total
	LOC	1.03×10^7	3.65×10^6	20.8
	Error	5.10×10^7	2.57×10^6	79.2

Table 2.2. Quantile regression fits to female size-fecundity relationships by shell condition for eastern Bering Sea snow crab pooled among years from 2007 to 2009. Parameter estimates and bootstrap estimated standard errors (SE) of the slope and intercepts of quantile regression fits to the 95% and 5% quantiles of the size-fecundity relationship. Data and linear fits are presented in Figure 2.4.

Quantile	95%		5%	
	Estimate	SE	Estimate	SE
Intercept	-77170	4305	-47770	15240
CW	2019	79	1217	283
SC3	-28640	8072	-14740	22840
SC4	-6183	12100	32670	26940
SC5	18720	38010	41080	33560
CW*SC3	709	140	28	473
CW*SC4	148	217	-871	430
CW*SC5	-334	729	-1082	611

Table 2.3. Results of linear mixed model analysis of variation in mean embryo weight by shell condition index (SC), year of collection (YEAR), an interaction term, and location of collection (LOC) estimated as a random component of the residual variance on mean embryo dry weight of female eastern Bering Sea snow crab with embryos in the early stages of development with from summer 2007 to summer 2009. Carapace width nested within shell condition and year was not a significant covariate (F = 1.36, $P = 0.18$) and was excluded from the final model. Results are summarized in Figure 2.4.

WHOLE MODEL FIT		N	R^2	RMSE
		853	0.52	5.53×10^{-6}
FIXED EFFECT TESTS		df	*F*	*p*
	SC	3	42.6	<0.0001
	YEAR	2	21.5	<0.0001
	SC*YEAR	6	12.2	<0.0001
COMPONENTS OF RESIDUAL VARIANCE		Variance	SE	% of Total
	LOC	0.005	0.0014	26.0
	Error	0.016	0.0008	74.0

Table 2.4. Results of linear mixed model analysis of variation in clutch dry weight by shell condition index (SC), year of collection (YEAR), carapace width (CW) nested within shell condition index and year, mean embryo dry weight (MEW) nested within shell condition index and year, and location of collection (LOC) estimated as a random component of the residual variance, for females with embryos in the early stages of development in the eastern Bering Sea from summer 2007 to summer 2009.

WHOLE MODEL		N	R^2	RMSE
		853	0.73	0.656
FIXED EFFECT TESTS		df	*F*	*p*
	CW[SC, YEAR]	12	76.6	<0.0001
	MEW[SC,YEAR]	12	4.09	<0.0001
	SC	3	16.6	<0.0001
	YEAR	2	4.78	0.044
	YEAR*SC	6	6.41	<0.0001
COMPONENTS OF RESIDUAL VARIANCE		Variance	SE	% of Total
	LOC	0.0055	0.0025	12.5
	Error	0.0375	0.0020	87.5

Table 2.5. Results of analysis of covariance (ANCOVA) of embryo quality and maternal characteristics for female snow crab, *Chionoecetes opilio*, sampled in the eastern Bering Sea in 2010. Embryo characteristics and embryo elemental composition were analyzed as a function of female carapace width (CW), shell condition (SC), and their interaction. The number of error degrees of freedom was 56 for each ANCOVA of embryo characteristics and 37 for embryo elemental composition. Significant factors (p<0.05) are indicated in bold.

Effects	df	*F*	*p*	df	*F*	*p*
	Fecundity			Mean Embryo Dry Weight		
CW	1	89.243	**<0.001**	1	0.811	0.372
SC	2	16.859	**<0.001**	3	15.165	**<0.001**
CW*SC	2	6.109	**0.004**	3	1.113	0.482
	Embryo Volume			% C (carbon)		
CW	1	1.793	0.186	1	0.019	0.890
SC	3	0.205	0.893	3	0.375	0.771
CW*SC	3	1.551	0.212	3	0.815	0.494
	% H (hydrogen)			% N (nitrogen)		
CW	1	0.054	0.818	1	0.780	0.383
SC	3	2.121	0.114	3	1.686	0.187
CW*SC	3	0.157	0.925	3	0.255	0.858
	C/N			C (µg/embryo)		
CW	1	0.941	0.338	1	0.212	0.648
SC	3	6.181	**0.002**	3	3.685	**0.020**
CW*SC	3	0.400	0.754	3	1.877	0.150
	N (µg/embryo)			H (µg/embryo)		
CW	1	0.537	0.468	1	0.003	0.957
SC	2	2.036	0.126	3	4.741	**0.007**
CW*SC	2	1.477	0.237	3	0.962	0.421

Table 2.6. Embryo characteristics and embryo elemental composition by shell condition for newshell (SC2), oldshell (SC3), and very oldshell (SC4) female snow crab, *Chionoecetes opilio*, sampled from the eastern Bering Sea in 2010. Values sharing the same letters were not significantly different (Tukey HSD, $p \geq 0.05$).

Embryo Characteristics			
Shell Condition	2	3	4
N	22	18	17
Embryo Volume (mm^3)	0.13 ± 0.00 a	0.13 ± 0.00 a	0.12 ± 0.00 a
Mean Embryo Dry Mass (µg)	68.2 ± 0.97 a	65.1 ± 1.32 ab	63.2 ± 1.24 b
Embryo Elemental Composition			
Shell Condition	2	3	4
N	16	10	15
% C	51.1 ± 0.36 a	51.4 ± 0.35 a	50.8 ± 0.41 a
% N	8.96 ± 0.08 a	9.18 ± 0.05 a	9.23 ± 0.06 a
% H	8.60 ± 0.29 a	7.64 ± 0.17 a	7.98 ± 0.26 a
C/N	5.71 ± 0.03 a	5.60 ± 0.05 ab	5.50 ± 0.02 b
C (µg/embryo)	34.4 ± 6.01 a	33.8 ± 7.90 ab	32.3 ± 7.19 b
N (µg/embryo)	6.04 ± 0.11 a	6.04 ± 0.13 a	5.86 ± 0.14 a
H (µg/embryo)	5.79 ± 0.21 a	5.03 ± 0.14 b	5.06 ± 0.17 b

Table 2.7. Correlation among indices of embryo quality for eastern Bering Sea snow crab, *Chionoecetes opilio*, sampled in the eastern Bering Sea in 2010. Pairwise Pearson correlation coefficient (*r*) and *p*-values for one-tailed t-tests among mean embryo weight (MEW), mean embryo volume (MEV), % C (carbon), % N (nitrogen), C/N (carbon/nitrogen ratio), and RES (residuals of the ANCOVA of fecundity are as a function of size and shell condition) are summarized, respectively, in the upper and lower triangles. Significant relationships ($p<0.05$) are indicated in bold.

	% C	%N	C/N	MEW	MEV	RES
% C	*	0.612	**0.353**	-0.019	-0.143	**0.399**
% N	<0.001	*	**-0.520**	-0.211	-0.182	0.259
C/N	0.024	<0.001	*	0.229	0.054	0.229
MEW	0.909	0.186	0.151	*	**0.357**	-0.122
MEV	0.374	0.256	0.737	0.008	*	-0.122
RES	0.010	0.102	0.447	0.365	0.367	*

Table 2.8. Size-fecundity relationships early versus late in the reproductive cycle for female snow crab collected from the eastern Bering Sea. Females were collected in the summer with egg clutches in early stages of development or in spring female by fisheries observers with egg clutches in the late (eyed) stages of embryo development. Analysis of covariance (ANCOVA) model effects include carapace width (CW) nested by shell condition index, year of embryo extrusion, and season (summer or spring), mean embryo dry weight (MEW) nested within the same terms as CW, and the nesting factors as main effects. Interaction terms were not significant (p>0.05) and were dropped from the model.

WHOLE MODEL FIT		N	df	F	p	R^2
		375	355	40.2	<0.0001	0.67
FIXED EFFECTS TESTS			df	F	p	
	CW [SC, YEAR, SEASON]		8	70.47	<0.0001	
	MEW [SC, YEAR, SEASON]		8	26.81	<0.0001	
	SC		1	7.60	0.0420	
	YEAR		1	0.05	0.8273	
	SEASON		1	1.74	0.1877	

Table 2.9. Results of linear mixed model analysis of size-fecundity by clutch fullness index (CFI) group, female carapace width (CW in mm), an interaction term, and location of collection (LOC) estimated as a random component of the residual variance with fecundity of female eastern Bering Sea snow crab collected from 2007 to 2009. Linear fits are shown in Figure 2.8A.

WHOLE MODEL FIT	N	R^2	RMSE
	853	0.80	469.6
FIXED EFFECT TESTS	df	*F*	*p*
CW	1	133.1	<0.0001
CFI	4	133.2	<0.0001
CW*CFI	4	13.7	<0.0001
COMPONENTS OF RESIDUAL VARIANCE	Variance	SE	% of Total
LOC	178.2	44.7	27.5
Error	469.8	23.9	72.5

Table 2.10. Weighted mean clutch fullness by shell condition class pooled among years (1994 to 2010) for eastern Bering Sea snow crab. Weighted mean CFI (± SE) and coefficient of variation (CV, ± SE) were not significantly different for groups sharing the same letter within each column (Dunn's post-hoc test; $p<0.05$).

	Weighted Mean CFI	CV of Weighted Mean CFI
SC2	4.8 ± 0.32 a	0.21 ± 0.017 a
SC3	4.6 ± 0.18 ab	0.30 ± 0.026 a
SC4	3.6 ± 0.35 bc	0.60 ± 0.059 b
SC5	2.8 ± 0.15 c	0.55 ± 0.50 b

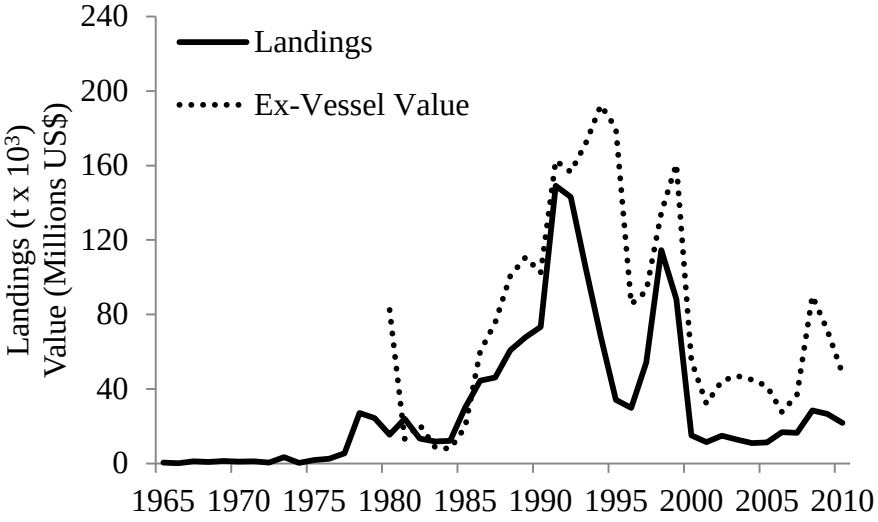

Figure 2.1. Value (nominal USD) and landings of eastern Bering Sea snow crab, 1965-2010.

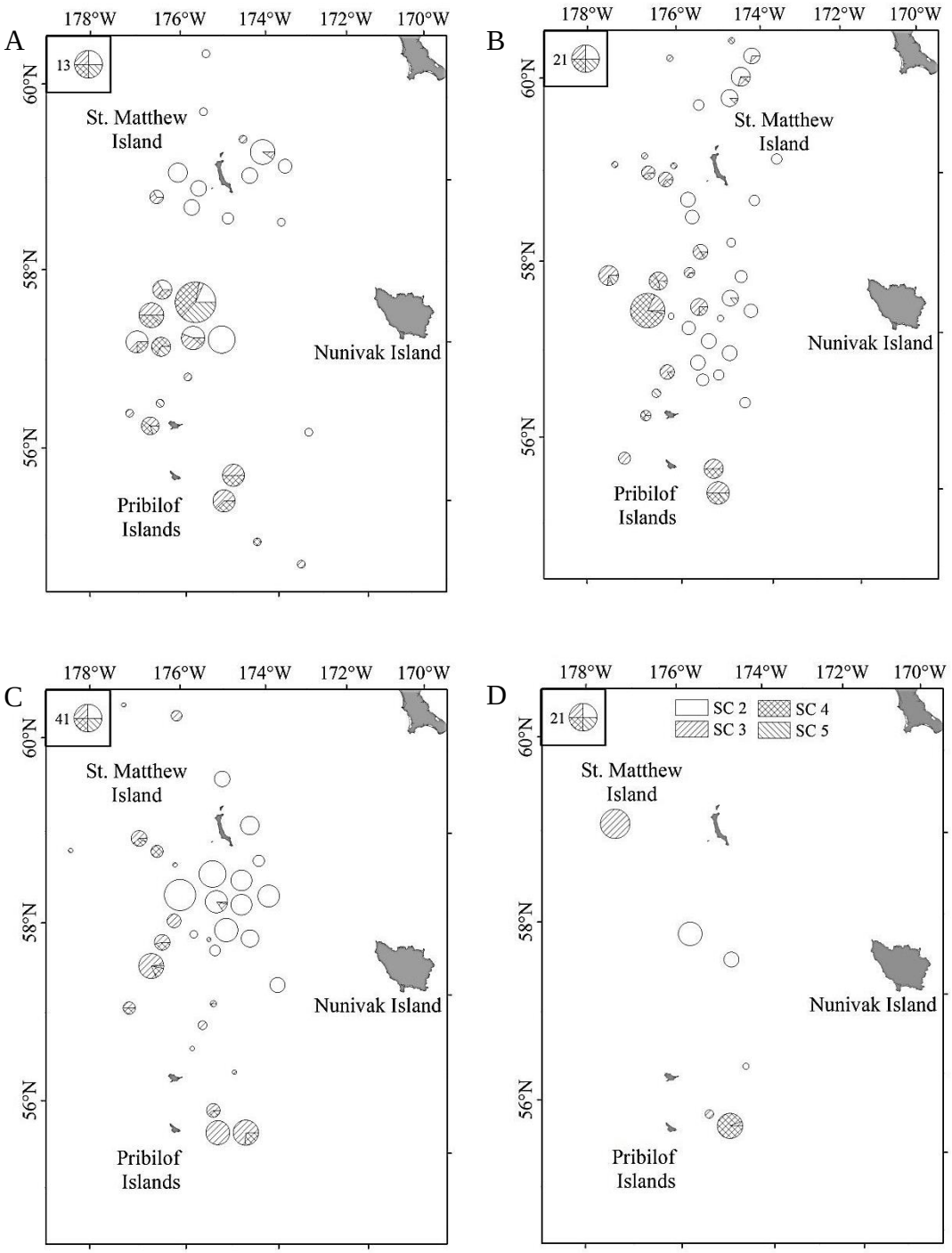

Figure 2.2. Map of sampling locations of female eastern Bering Sea snow crab by shell condition group collected during annual summer bottom trawl surveys for investigation of size-fecundity in A) 2007, B) 2008, and C) 2009; D) embryo quality in 2010. Symbol size (top left of each panel) and symbol hatching (key in panel D) indicate, respectively, the number of females collected and shell condition composition at each location sampled.

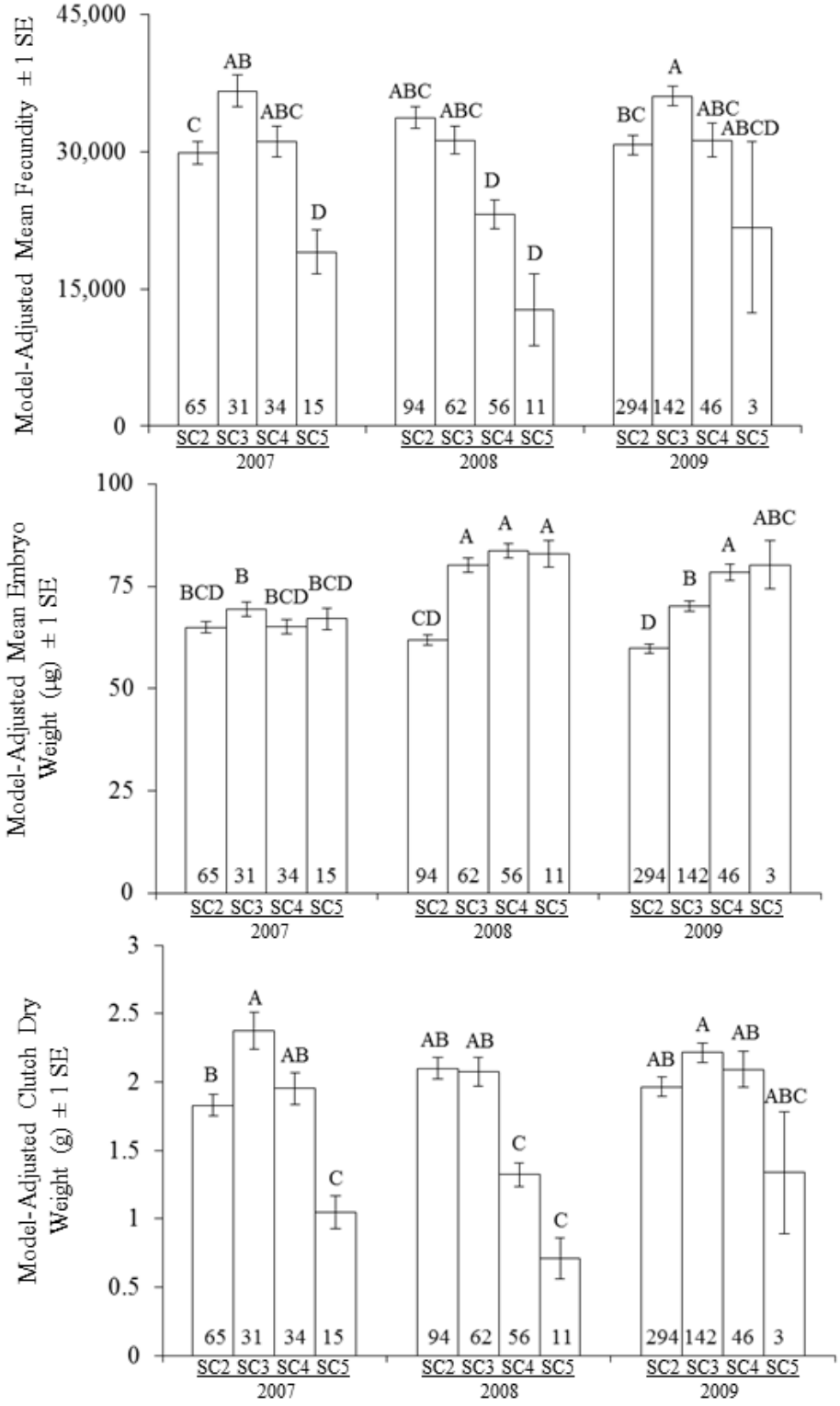

Figure 2.3. Model adjusted fecundity, clutch dry weight, and mean embryo weight of sampled mature female snow crab from the eastern Bering Sea (n=853) with embryos in the early stages of development by year (2007, 2008, 2009) by shell condition index (SC2 – Newshell, SC3- Oldshell, SC4- Very Oldshell, SC5 presented as mean (mean ± SE). Levels sharing the same letter were not significantly different (Tukey HSD; $p<0.05$).

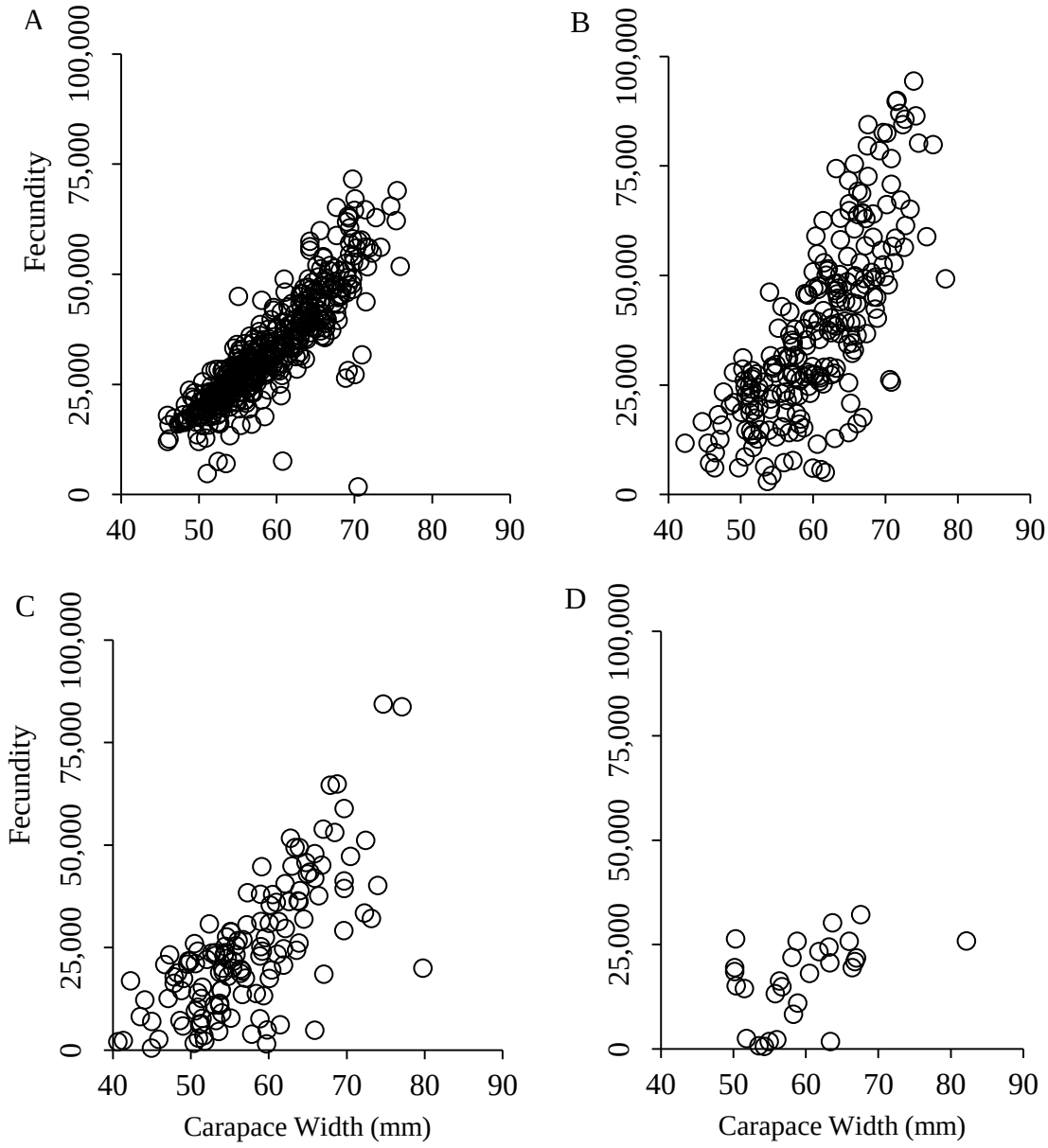

Figure 2.4. Quantile regression fits of size-fecundity by shell condition group (solid black lines) at the 95th (upper line) and 5th (lower line) for SC2 – Newshell (A), SC3-Oldshell (B), SC4- Very Oldshell (C), and SC5 – Very, Very Oldshell (D) mature female snow crab with egg clutches in the early stages of embryo development collected from the eastern Bering Sea from 2007 to 2009.

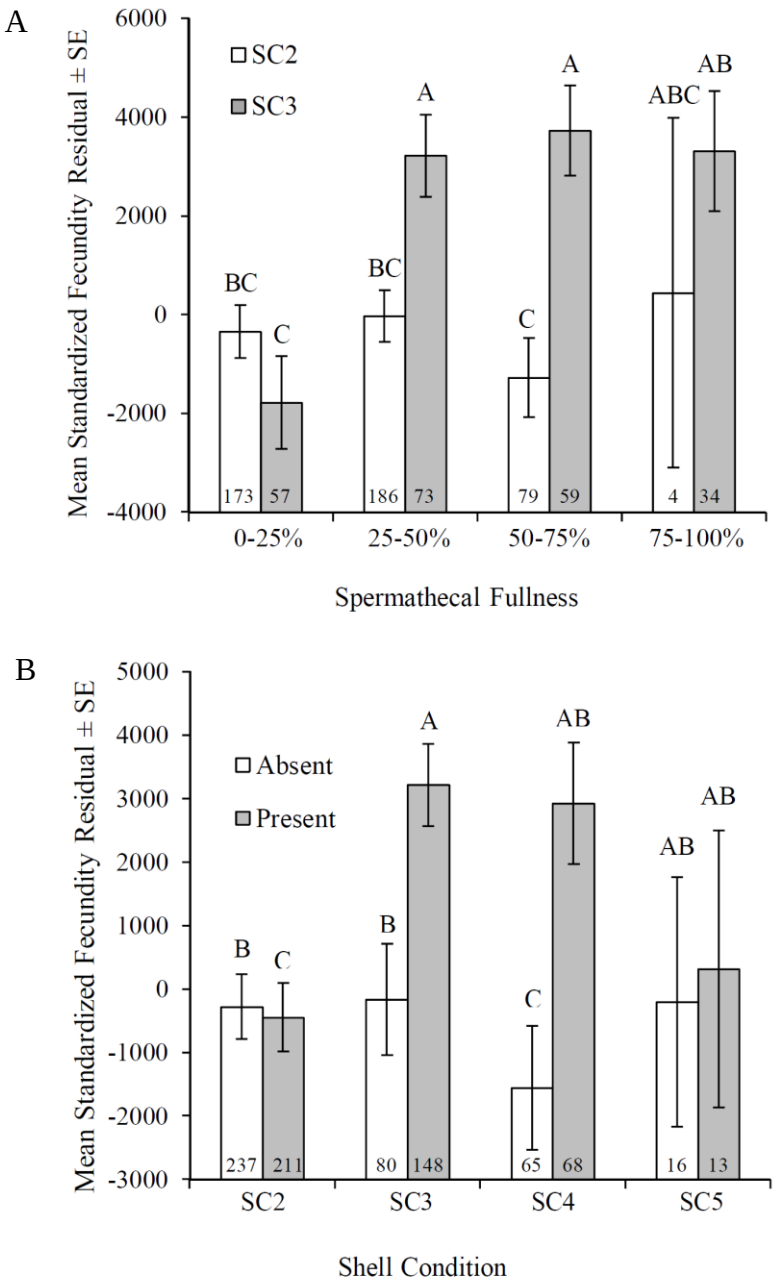

Figure 2.5. Mean standardized fecundity residuals and sperm reserves, indexed as the qualitative assessment of spermathecal fullness (A) or the presence-absence of fresh ejaculate (B), by shell condition group for mature female snow crab sampled from eastern Bering Sea from 2007 to 2009. Residuals (mean ± SE) were standardized by linear mixed model and levels sharing the same letter were not significantly different (Tukey HSD; $p<0.05$).

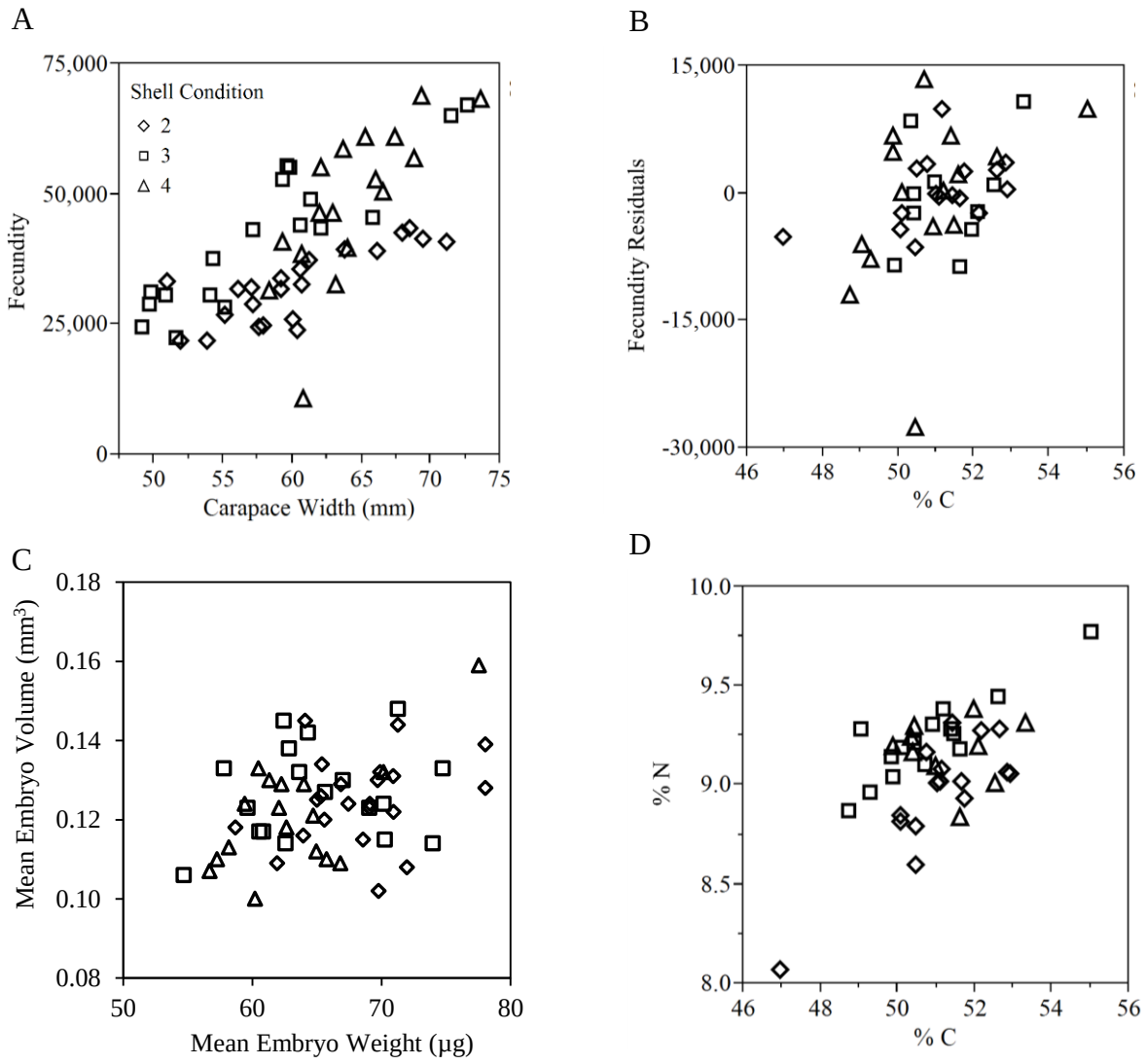

Figure 2.6. Size-fecundity and relationships among indicators of embryo quality by shell condition for female snow crab collected for investigation of embryo quality in the eastern Bering Sea in 2010 including (A) Size-fecundity relationship (B) Residual fecundity (standardized for maternal size and shell condition) and embryo % C, (C) mean embryo volume and mean embryo weight, and (D) embryo % C and embryo % N

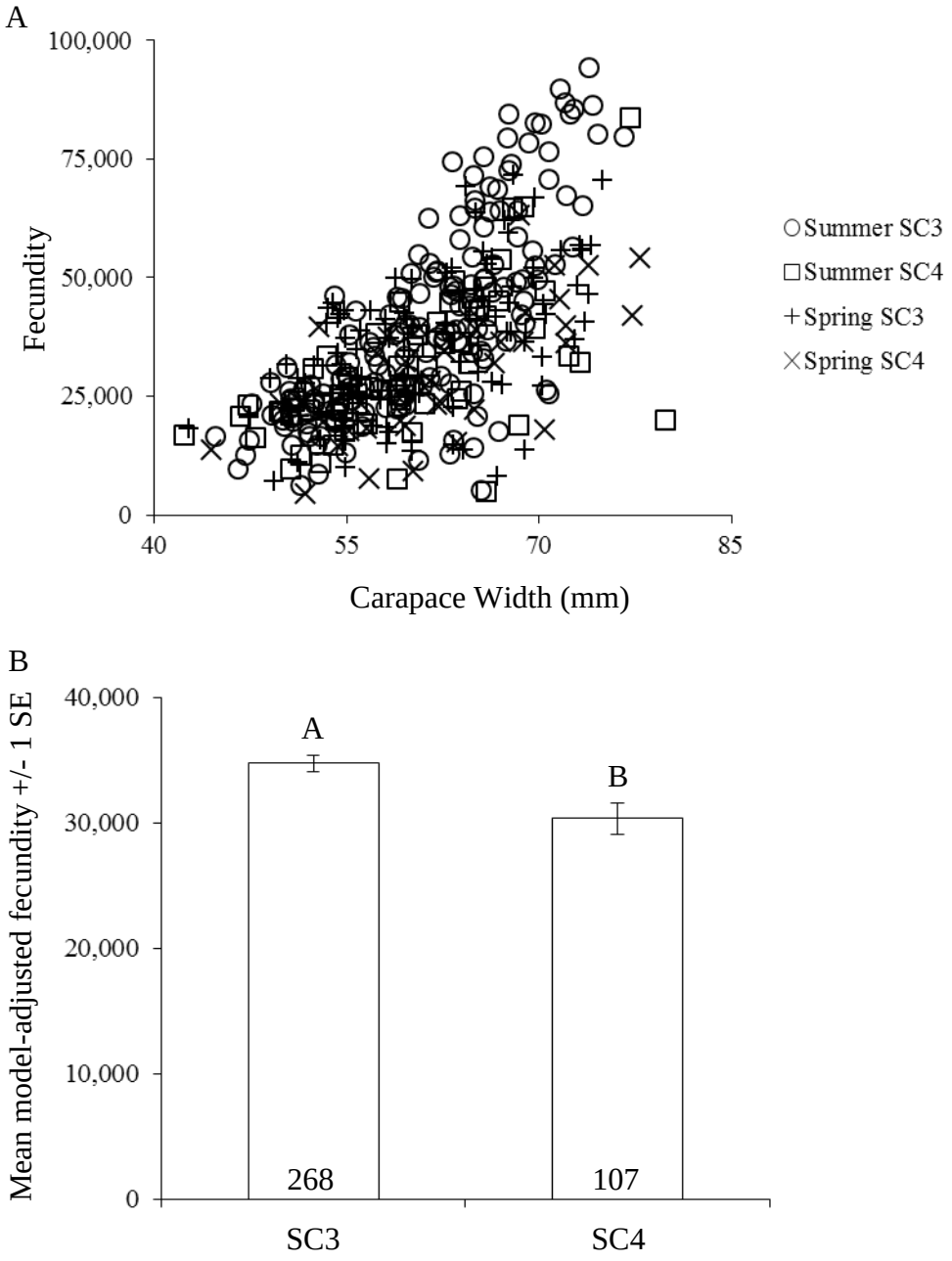

Figure 2.7. Size-fecundity early versus late in the reproductive cycle for female eastern Bering Sea snow crab collected in the collected from the eastern Bering Sea in summer 2007 and 2009 while brooding embryos in the early stages of development or with embryos in the late (eyed) stages of embryo development in spring 2008 and 2010 is shown in panel A and mean model-adjusted (ANCOVA) fecundity of SC3 and SC4 pooled among years and seasons is shown in panel (B) Summer samples were collected during the NMFS bottom trawl survey and spring samples SC3 (oldshell) and SC4 (very oldshell) female snow crab (n=375) were collected during groundfish fisheries by onboard observers. Sample sizes are indicated within the bars and levels sharing the same letter were not significantly different (Tukey HSD; $p<0.05$).

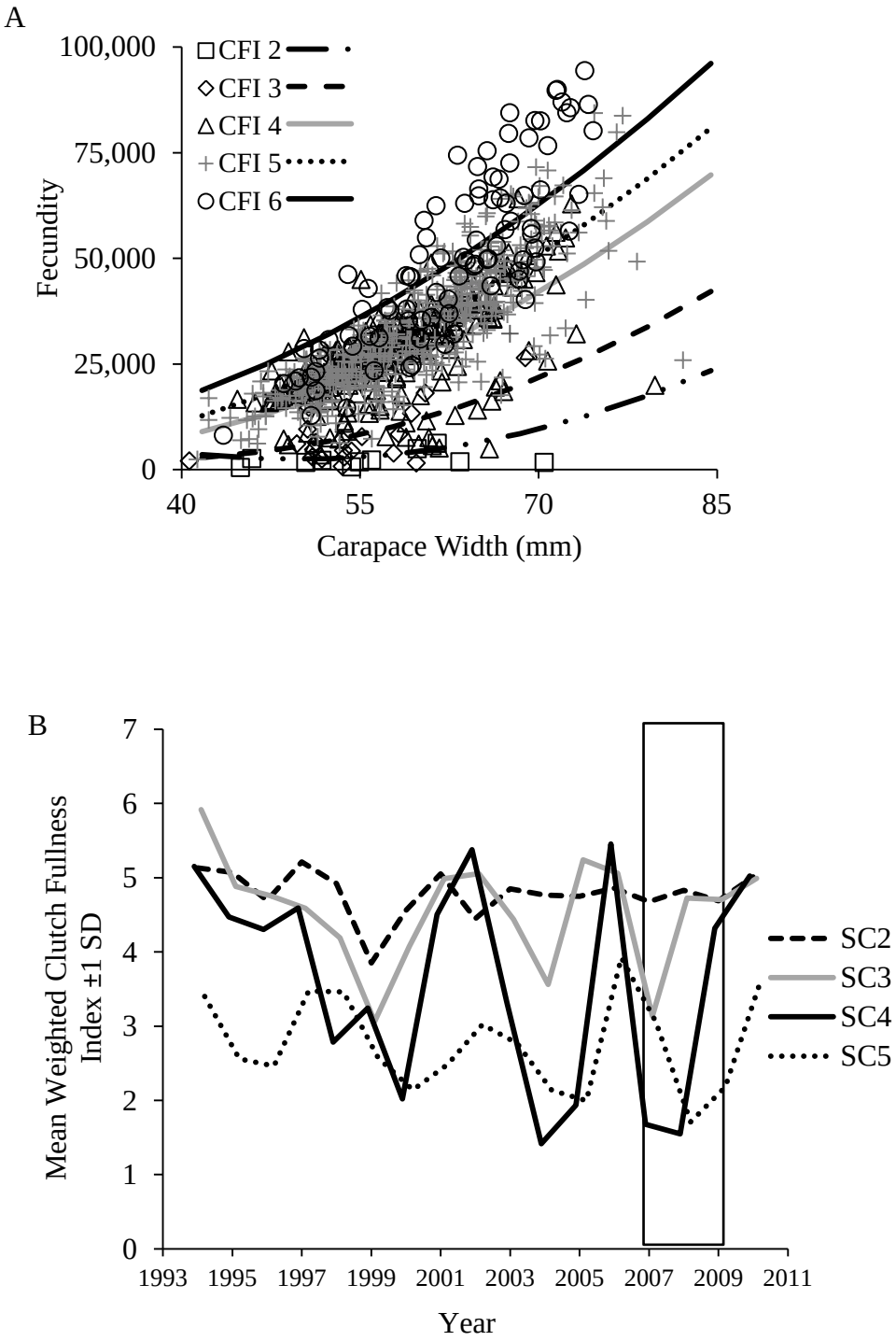

Figure 2.8. Size-fecundity relationships and survey time series of clutch fullness index (CFI). (A) Best-fit lines by CFI category estimated by ANCOVA (Table 10) for females collected for investigation of fecundity from 2007 to 2009. B. Interannual (1994-2010) variation in weighted mean clutch CFI by shell condition index for mature female snow crab from eastern Bering Sea annual stock assessment surveys (Chilton et al. 2011). Years of investigation of female fecundity (2007-2009) are boxed for reference.

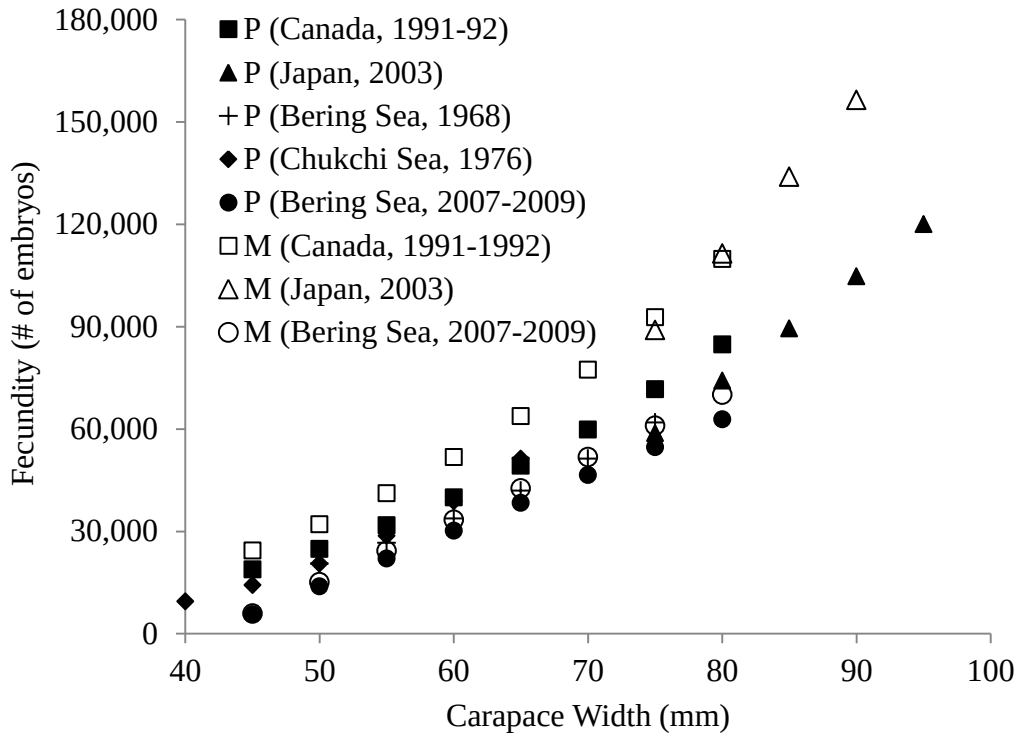

Figure 2.9. Best-fit size-fecundity relationships among snow crab stocks based on literature values and evaluated at 5 mm intervals of female carapace width (mm) for primiparous (P) and multiparous (M) females from the northwest Gulf of Sainte Lawrence, Canada (Sainte-Marie 1993), Wakasa Bay, Japan (Kon and Adachi 2006), southeastern Bering Sea (Haynes et al. 1976, this study), and Chukchi Sea (Jewett 1981).

Chapter 3

The effect of demography on reproductive potential and implications for recruitment of eastern Bering Sea snow crab, *Chionoecetes opilio*[1]

Abstract

Snow crab, *Chionoecetes opilio*, in the eastern Bering Sea (EBS) support an important commercial fishery managed with large-male-only harvest and biological reference points determined from mature male biomass. However, mature male biomass may be a poor proxy for female reproductive potential and stock productivity. We developed egg (embryo) production indices that incorporate information on female demography and fecundity into estimates of stock egg production to better inform management decisions. Both male and female indices of abundance were positively correlated with egg production, but stronger association was observed with female versus male indices. Estimates of annual egg production also decreased with the incorporation of detailed demographic and size-fecundity information, inferring up to ~50% lower annual egg production than a simple index based on average fecundity. First-time reproducing (primiparous) females frequently occupied waters with bottom temperatures <0° C, while females reproducing for the second or more (multiparous) were primarily found in warmer waters. Efforts to infer the fraction of biennially reproducing females from average conditions based on near-bottom temperatures and fluctuations in abundance were hindered by high variability in the underlying data. A positive relationship between reproductive potential and recruitment, defined as instar vii juveniles (25-33 mm carapace width), was evident at a lag of 4 yr. This relationship was primarily driven by brood years in the late 1980s and was more robust for indices based on mature females and egg production than those based on males. A decline in stock productivity (ln R/S) in the early 1990s preceded the dramatic decline in EBS snow crab abundance in the mid to late 1990s with an increasing trend in in stock productivity thereafter.

[1] Webb, J.B, G.H. Kruse, and L.M. Stichert. The effects of demography on female reproductive potential and implications for recruitment of eastern Bering Sea snow crab, *Chionoecetes opilio*. Prepared for submission to Fisheries Research.

3.1 Introduction

Marine fisheries management is frequently challenged by a limited understanding of the complex life history and ecological factors that influence population regulation and persistence (Houde, 2008). Broad correlations between indices of reproductive potential (e.g. spawner abundance) and recruitment have been observed for marine fish with diverse life history characteristics (Myers and Barrowman, 1996), but robust functional relationships are rare for crabs and lobsters (Caddy, 1986; Wahle, 2003). However, environmental variability, larval dispersal, metapopulation structure, and complex demographic effects can substantially modify relationships between reproductive potential and recruitment for both fish and invertebrates (Caputi et al., 2003; Cowen and Sponaugle, 2009; Myers, 1998). Understanding of the cumulative effects of these factors on population renewal processes has been advanced by (1) development of refined estimates of reproductive potential that incorporate variability in spawning stock demography (Kraus et al., 2002; Marshall et al., 2009; Morgan et al., 2011); (2) use of coupled biophysical models to estimate connectivity and define metapopulation structure (Incze et al., 2010; Xue et al., 2008); and (3) analyses that consider multiple life stages and both intrinsic and extrinsic factors affecting stock reproductive potential, survival by life stage, and recruitment (Hidalgo et al., 2011; Hinckley et al., 2010; Lipcius and Stockhausen, 2002).

During peak harvests from 1990 to 1998 the eastern Bering Sea (EBS) stock of snow crab, *Chionoecetes opilio*, supported one the largest crab fisheries in the world. However, a dramatic decrease in abundance in the late 1990s led to an "overfished" designation under US fishery regulations and adoption of a rebuilding plan, including measures designed to reduce fishing mortality and conserve stock reproductive potential. Fishery management is currently implemented through large-male-only harvest and catch quotas that cannot exceed a maximum fishing mortality rate associated with overfishing *(F_{OFL}).* For EBS snow crab, when biomass exceeds B_{MSY}, the theoretical mean biomass that yields maximum sustainable yield, F_{OFL} is set to the F_{MSY} proxy of $F_{35\%}$, the fishing mortality that would reduce the fertilized embryo production per recruit to 35% of the unfished stock under equilibrium conditions. When biomass < B_{MSY}, then F_{OFL} is determined as a declining linear function of stock biomass until a minimum stock size threshold is reached, below which no catch is allowed. Due to a paucity of information on female reproduction, "stock biomass" in the harvest control rule is defined to be mature male biomass estimated by a length-based assessment model (Turnock and Rugolo, 2013).

High variability in recruitment, biomass, and harvest are common to exploited snow crab stocks in the US and Atlantic Canada; both intrinsic and extrinsic mechanisms may contribute to this variability (Kruse et al., 2007; Orensanz et al., 2004; Sainte-Marie et al., 1996, 2008). Investigations of life history, spatial dynamics, and ecological context for female EBS snow crab have furthered understanding of factors likely to contribute to variability in cohort strength (Burgos et al., 2013; Ernst et al., 2005, 2012; Parada et al., 2010; Zheng and Kruse, 2006). Briefly, life history and spatial dynamics are strongly associated with regional oceanography. The EBS shelf consists of three bathymetrically defined domains: coastal (0-50 m), middle (50-100 m), and outer (100-200 m) each with distinct oceanographic characteristics (Stabeno et al., 2001). Juvenile EBS snow crab are strongly associated with the "cold pool", an area of the middle domain with near-bottom water temperatures <2° C during the spring and summer (Ernst et al., 2005; Orensanz et al., 2004). The spatial extent of this water mass during summer is directly related to maximum sea ice extent the previous winter (Zhang et al., 2012). Female snow crab are thought to undergo their terminal (final lifetime) molt to maturity in the middle domain and subsequently move in a south/southwest direction into the deeper, warmer waters of the outer domain (Ernst et al., 2005; Parada et al., 2010; Zheng et al., 2001). The geographic range of mature female EBS snow crab contracted northward in the 1980s coincident with a northward retreat of the cold pool (Mueter and Litzow, 2008; Orensanz et al., 2004). Southward expansion is likely to be hindered by directional south to north oceanographic connectivity, increased predation by Pacific cod (*Gadus macrocephalus*) in the southern portions of the snow crab range, and predicted long-term warming (Burgos et al., 2013; Orensanz et al., 2004; Parada et al., 2010; Zheng and Kruse, 2006).

Potential determinants of recruitment variability have been extensively investigated for EBS snow crab. Recruitment of female snow crab to the mature stock is cyclical, occurring with 6-7 yr periodicity in the middle domain, an interval coincident with the duration from embryo extrusion to first reproduction (Ernst et al., 2012). This pattern, along with higher mean potential for larval retention in the middle domain based on larval drift modeling, suggests that the abundance of females, primarily primipara (completing the first reproductive cycle), releasing larvae in the middle domain may be most important to future recruitment (Burgos et al., 2013; Ernst et al., 2012; Parada et al., 2010). Food availability during the larval phase, settlement

location, and temperature at settlement location were the most important factors determining survival to recruitment and modeled recruitment levels were correlated (best model r^2=0.54) with estimates from the length-based assessment model (Hinckley et al., 2010). Retrospective analyses of relationships among reproductive potential, environmental factors, and recruitment suggest a temporal shift in control of juvenile recruitment from intrinsic (female reproductive potential) to extrinsic factors coincident with a "regime-shift" in 1989 from relatively warm to relatively cool conditions indexed by the Pacific Decadal Oscillation (Szuwalski and Punt, 2013).

Varying mature female demography and induction of biennial reproduction at cold temperatures are other potentially important sources of variability in reproductive potential. Female fecundity changes with reproductive ontogeny; maximum female fecundity is observed during the first few reproductive cycles and declines thereafter as female become senescent (Webb et al., Chapter 2 this dissertation). Variabilty in average clutch fullness index (CFI, categorical visual evaluation of size of the egg clutch) reflects this pattern with decreasing CFI associated with increasing age of mature female EBS snow crab relative to the terminal molt (Ernst et al., 2012, Orensanz et al., 2005). Cold bottom water conditions (< ~ 0-1 ° C) have synergistic effects on female reproductive potential through decreased female size-at-maturity (Orensanz et al., 2007) and decreased tempo of reproduction as the duration of embryo development is extended from 1 to 2 yr (Kuhn and Choi, 2011; Moriyasu and Lanteigne, 1998; Sainte-Marie et al., 2008; Webb et al. 2007).

Improved knowledge of variability in stock reproductive potential is a critical factor for improved management of the EBS snow crab fishery, as it could allow the derivation of stock-recruit relationships from which to reliably estimate F_{msy} and its probability density function, necessary to apply improved harvest control rules that are not reliant on the use of $F_{35\%}$ as a proxy for F_{MSY}. Best fit stock-recruit relationships previously estimated for EBS snow crab vary in functional form (e.g. Ricker or Beverton-Holt) with the choice of lag from oviposition (egg extrusion and fertilization) to recruitment and index of reproductive potential employed (Szuwalski and Punt, 2013; Zheng and Kruse, 2003; Zheng and Kruse, 2006). High levels of autocorrelation in the residuals and large variability in recruitment at both high and low levels of reproductive potential led Zheng and Kruse (2006) to conclude that density-independent factors

are likely to be most influential in snow crab recruitment. Environmental factors, predation, or density-dependent factors including growth variability, aging error, variability in fecundity per unit of spawning stock, or spatial variability in reproductive output per unit spawning stock may mask underlying stock-recruit relationships (Caddy, 1986; Caputi, 1993; Zheng and Kruse, 2006). With this in mind, the goal of our study is to integrate knowledge of the complex factors governing reproductive potential into a refined index of stock reproductive potential by achieving three objectives: (1) investigate development of formal methods to incorporate annual/biennial reproduction and size/shell condition specific fecundity into estimates of annual egg production; (2) compare indices of reproductive potential incorporating varying levels of demographic information; and (3) evaluate possible relationships between refined indices of female reproductive potential and recruitment.

3.2 Methods and Materials

3.2.1 Study domain and data source

The spatial domain of this study is the portion of the EBS shelf covered by the National Marine Fisheries Service (NMFS) bottom trawl survey for crab and groundfish (Fig. 3.1), which has been conducted annually, primarily during June and July, since 1978 (Daly et al., 2014). Standard survey stations are generally located near the center of 20 X 20 nm grid cells with the addition of "corner stations" at the grid intersections in regions of high crab density near the Pribilof Islands and St. Matthew Island. The trawl survey dataset used for this analysis spanned 1978 to 2012 (obtained from Bob Foy, National Marine Fisheries Service, Kodiak Laboratory). The geographic extent and number of stations surveyed within each year varied due to the progressive addition of stations to the eastern and northern boundaries of the survey area between 1978 and 1988 (Daly et al., 2014). Biological data available from the survey database included carapace width (CW, measured to the nearest millimeter), shell condition (SC2 to SC5) for all crab; egg clutch color (orange, purple, brown, purple-brown, pink), egg condition (uneyed, eyed, dead eggs, egg cases), maturity determination based on the width of the abdomen, and CFI (1- no eggs, 2- trace eggs, 3- 25%, 4-50%, 5-75%, and 6-100% clutch fullness) for mature females (Jadamec et al., 1999; Orensanz et al., 2005). Male and female age relative to the terminal molt is indexed by a shell condition index determined from relative changes in wear and epibiont load of the exoskeleton into newshell (SC2), oldshell (SC3), very oldshell (SC4), and

very, very oldshell (SC5) categories (Ernst et al., 2005; Fonseca et al., 2008; Jadamec et al., 1999). Near-bottom temperature measured to the nearest 0.1 °C is also available for >95% of the stations in the database.

3.2.2 Biennial reproduction

A stage-based model structured by female shell condition was developed and fitted to trawl survey indices of abundance to derive estimates of average survival and stage transition parameters (Appendix, Figure 3.2a). Parameter estimates were then used to iteratively calculate the proportion of females likely to be carrying egg clutches in the intermediate stages of oviposition (i.e. not hatching or undergoing oviposition in the current year) during the summer of the current year based on the proportion of female abundance distributed in strata defined by bottom trawl survey stations with cold, near-bottom seawater temperatures (indexed in this study by the 0, 0.5, or 1° C isotherms) likely to induce biennial reproduction (Appendix 3.A).

3.2.3 Indices of reproductive potential and recruitment

Indices of egg production, measured as embryo production, incorporating successively greater levels of available biological information were calculated to gauge the effect of each factor on estimated reproductive potential. Fecundity estimates used in calculation of embryo production were obtained by: (1) shell condition group, (2) size (5 mm CW bins) and shell condition group, and (3) size and CFI (1-6; Orensanz et al., 2005) group from a multi-year study of snow crab fecundity in the EBS (Webb, Chapter 2 this dissertation). The indices of egg production were: (1) constant fecundity, estimated as the product of area-swept mature female abundance and mean fecundity of all mature females, (2) fecundity by shell condition, the product of area-swept abundance by shell condition class and average fecundity by shell condition for each year, and (3) four seasonal indices of annual egg production were estimated with varying sets of assumptions. First, determinate estimates of annual summer egg production (EPI_t^{SM}) were combined into a time series of summer egg prouduction (summer EPI-B, egg production proximate to oviposition) incorporating biennial reproduction, female size-frequency, and CFI. EPI_t^{SM} was estimated as:

$$EPI_t^{SM} = \sum_i^n \sum_j^n \sum_k^n \left(AI_{i,j,k,t} - \left(AI_{i,j,k,t} \cdot P_{i,t}^B \right) \right) \bar{F}_{j,k} \quad (1)$$

where $AI_{i,j,k,t}$ is the abundance index by shell condition (i), CFI (j), carapace width bin (k), and year (t); $\bar{F}_{j.k}$ is the fecundity of a female of CFI class j at the midpoint of carapace width bin (k) by 5 mm bins from 36 mm to 90 mm CW; and $P_{i,t}^{B}$ is the proportion of females inferred to reproduce biennially (Appendix 3.A). Second, to examine the influence of biennial reproduction on EPI, an additional index (summer EPI) was calculated excluding the fraction biennial term ($AI_{i,j,k,t} \cdot P_{i,t}^{B}$). Finally, two refined indices of egg production, also excluding biennial reproduction, were estimated based on geographic criteria were estimated by eq. 5 to reflect the hypothesized importance of the reproductive contribution of females from these areas to the stock renewal process: 1) egg production of females in the middle domain of the EBS (Figure 1), and 2) egg production of females in the central and southern middle domain (Figure 1).

Three additional spawning stock abundance indices were estimated by the area-swept method from the trawl survey data: mature female abundance, mature male abundance, and adult male abundance. Mature females were identified by a disproportionately large abdomen relative to immature females, mature males as all males with CW > 40 mm, and adult males as those likely to have completed the terminal molt to maturity defined by large chelae relative to body size. Male snow crab achieve sexual maturity (spermataphore production) at small body size (~40 mm CW) relative to the range of body sizes over which the terminal molt is observed. When males molt to adulthood, their chela height becomes disproportionately large relative to body size as compared to those who have not terminally molted (Sainte-Marie et al., 1995). Terminally-molted, large-claw males may exclude non-adult males under competitive mating conditions (Sainte-Marie et al., 2008). Using a published description of the proportion of EBS males which were large-claw by carapace width bin (Otto 1998), we developed a step function to estimate the proportions of large-claw males in 20 mm CW bins: 0.20 at 40-60 mm CW, 0.45 at 61-80 mm CW, 0.55 at 81-100 mm CW, 0.80 at 101-120, and 1.00 at >121 mm CW.

Estimates of juvenile recruitment (~25-40 mm CW) were obtained from a length-based model as reported in the annual stock assessment (Table 6 in Turnock and Rugolo, 2013). Alternative recruitment indices were also estimated for EBS snow based on size-at-instar structure (Ernst et al., 2012) as the area-swept abundance of instar vii (25-33 mm CW), likely ~3-4 yr post-settlement and instar viii (34-43 mm), likely 4-5 yr post-settlement (Comeau et al., 1998; Ernst et

al., 2012; Kilada et al., 2012). Size ranges of males and females at these instars is similar those for snow crab in other regions (Comeau et al., 1998).

3.2.4 Statistical analyses

The stage-based model was fitted by least-squares and profile confidence intervals of parameters were obtained using PopTools v. 3.2.5 (www.poptools.com) in Excel 14.0. Where bottom temperature data were missing for stations in the trawl survey database these values were interpolated from the observed values at surrounding stations using a natural neighbor algorithm in ArcGIS 10.2.2 (ESRI Inc. Redlands, CA). The relative influence of model error versus female distribution in cold versus warm water on the inferred proportion biennial was examined by non-parametric (Spearman's) partial correlation. Associations among time-series of spawning stock/egg production indices and among recruitment indices were evaluated by Pearson correlation coefficients (r). Association among recruitment indices at varying time lags was investigated by cross-correlations. Prior to correlation analysis, time-series were evaluated for stationarity using the augmented Dickey-Fuller test and autocorrelation at lag k by the Ljung-Box Q test. Non-stationary series were first-differenced prior to comparison; when significant autocorrelation was indicated, the effective sample size (N^*) was determined for each comparison by the modified Chelton method using autocorrelation lags of $n/5$, where n is the length of the time-series (Pyper and Peterman, 1998). A non-parametric one-way test (Kruskal-Wallis) and post-hoc multiple comparisons test (Dunn) were used to evaluate differences in the proportion females in cold versus warmer waters by threshold near-bottom temperature and shell condition class. Time-series, correlation, and one-way analyses were conducted in JMP 10.1 (SAS Institute, Cary, NC). A threshold of significance level of $\alpha=0.05$ was used for all analyses. Functional relationships between indices of reproductive potential (S) and recruitment (R) were fitted by optimization using the FLR package in R 2.15.13 (R Development Core Team, 2013) assuming log-normally distributed recruitment. Standard deviations, used as indices of uncertainty of the model parameters, were obtained from the variance-covariance matrix. Evidence of an observable functional relationship between S and R was evaluated by fitting models presuming no relationship (mean recruitment) up to higher order two-parameter stock-recruitment models. Model fits to the data were assessed based on ΔAIC_c (Wang and Liu, 2006) and root mean squared error (RMSE). Models with $\Delta AIC_c \leq 2$ were considered competitive and models with $\Delta AIC_c \leq 4$ were considered plausible. Mean recruitment ($\bar{R}=\beta$), density-independent

($R=\alpha S$), Ricker ($R=\alpha S e^{-\beta S}$), and Beverton-Holt ($R=\alpha S/\beta+S$) models were fitted to each combination of reproductive indices versus recruitment. First-order autocorrelation of the model residuals was evaluated graphically (residuals at lag 0 versus lag +1) and by examination of ΔAIC_c when fitting the model with and without the first-order autocorrelation coefficient rho *(ρ)*. The absence of a stock-recruit relationship was presumed when mean recruitment was identified as the best fit (i.e. sole competitive) model based on ΔAIC_c.

3.3 Results

3.3.1 Stage-based model

The stage-based model fit over 1989 to 2012 was chosen as the best model based on consistency in survey gear and spatial extent of the survey, duration of the survey time-series included in the model, graphical evaluation of agreement between model and survey indices of abundance (Fig. 3.2b). Model-estimated abundance indices generally agreed with the observed data during the 1990s when a strong recruitment pulse to the newshell class was observed and tracked through the old and very oldshell classes, but agreement between observed and fitted indices was lower and lagged progression of cohorts by stage was not evident in subsequent years (Fig 3.*2*c). Variation in estimated indices of abundance of oldshell and very oldshell females was lower (i.e. smoothed) in comparison to area-swept estimates (Fig. 3.2c). Oldshell female abundance estimated from the stage-based model was lower than the area-swept estimates from 1989 to 1992 and the model-estimate of $N_{0,v}$ (1989) was lower than the observed value (Fig. 3.2b). Estimates of survival ($\hat{S}$) and the proportion of females remaining at the oldshell stage ($\hat{P}_o$) from the stage-based model varied with fitted time period (Table 3.1). Estimated values of P_n consistently converged to the lower bound of near zero in the full model (all parameters estimated), so this parameter was fixed at zero to fit the final model. Likelihood profiles suggested that model fits were more sensitive to variability in $\hat{S}$ than $\hat{P}_o$ (Fig. 3.2c). Using parameter estimates from the best model, a simple extrapolation of a cohort from recruitment to the newshell stage in year *x* to extinction at the very oldshell stage indicated that >95% of the cohort would progress into the very oldshell class at 5 yr post-recruitment, <5% of the cohort would remain in the very oldshell class at 7 yr post-recruitment, and <1% to 9 yr.

3.3.2 Association with the cold pool and biennial reproduction

Mean proportion of the survey area covered by cold water during the study period ranged from 0.12 at 0° C to 0.21 at 1° C (Fig 3.3a). The proportion also varied interannually with low values, indicative of warmer than average conditions, during the first few years of the time series, higher values in the early 1990s relative to the 1980s, and a distinct peak in 1999. Cold near-bottom water was scarce from 2000 to 2005 but expanded again from 2006 to 2010 (Fig. 3.3a). At 0.5 °C a significantly (Kruskal-Wallis; $p<0.0001$; $\chi^2_{2,103} = 48.5$) greater proportion (mean ± SD) of newshell female abundance (0.50 ± 0.32) (Dunn's post-hoc test, $p<0.0001$) was observed in cold water than for oldshell (0.09 ± 0.11) or very oldshell (0.04 ± 0.08) females, which were not significantly different (Fig. 3.2a).

Coherent with patterns of association with cold water among females of varying shell condition, the inferred proportion (mean ± SD) of females reproducing biennially was 0.41 ± 0.80 and 0.05 ± 0.11 at 0 ° C, 0.48 ± 0.83 and 0.06 ± 0.12 at 0.5° C, and 0.64 ± 1.06 and 0.18 ± 0.45 at 1° C respectively, for oldshell and very oldshell females (Fig. 3.3b). However, biased values (greater than 1.0) were evident for the proportion of oldshell females inferred to reproduce biennially at all threshold temperatures and for very oldshell females at 1° C (Fig 3.3b). The frequency and magnitude of biased values increased with increasing threshold temperature from 0 to 1° C (Fig. 3b). The pattern of partial correlation (Spearman's rho) among variables suggested that bias was due to the large deviations (residuals) between the observed abundance of oldshell females in cold near-bottom temperatures versus those expected under average conditions (i.e. model-estimated). Partial correlations between the factors expected to influence the inferred proportion biennial, and the proportions of newshell (0.25, $p=0.15$) and oldshell females (0.20, $p=0.25$) in cold water (<0.5 °C) in year *t*-1, were not significant. On the other hand, the partial correlation was -0.73 ($p<0.001$) between the inferred proportion of biennially reproducing oldshell females and the deviation (e.g. residual) between the inferred abundance of oldshell females reproducing biennially (based on model parameter estimates) and the area-swept abundance of oldshell females in year *t*. Due to bias in the inferred proportion of females reproducing biennially, further analyses of egg production did not include adjustment for biennial reproduction.

3.3.3 Egg production and indices of reproductive potential

Refining estimates of reproductive potential with female demography and fecundity resulted in substantial decreases in cumulative egg production compared to simpler indices. Constant fecundity, fecundity by SC, and summer egg production indices tracked closely prior to the late 1980s, but the summer and summer-B indices trended below the simpler indices from the late 1980s onward (Fig. 3.4a). Temporal variability in egg production of females in the middle domain and southern middle domain was characterized by three cycles with peaks in the early and late 1980s, mid 1990s and an additional peak in the early 2000s that was observed in the middle domain index but not southern middle domain (Fig 3.4a).

Indices of reproductive potential based on aggregate egg production, mature female, mature male, and adult male abundance were significantly and positively correlated; Pearson correlation coefficients among first-differenced indices ranged from 0.63 to 0.97 (Table 3.2). The index of summer egg production was robustly correlated with female abundance ($r \geq 0.97$; Table 3.2) and the lowest correlations were observed between indices of egg production and male abundance (r =0.68; Table 3.2). While the correlation between egg production and mature female abundance was high (0.97, Table 3.2, Fig. 3.3), important differences were also observed in the magnitude of estimated egg production among indices within a year (Fig. 3.3a). The difference between summer EPI and CF (mature female abundance scaled by average female fecundity) ranged from +5% in 1988 to -55% in 1998 with a mean of 22%. The strength of correlation between egg production of females in the middle domain and southern middle domain and indices of aggregate egg production and male and female abundance was variable, ranging from 0.30 to 0.76 (Table 3.2). Egg production in the southern middle domain was significantly correlated with both summer and middle domain egg production but not with any of the indices of mature abundance.

3.3.4 Recruitment indices and stock-recruit relationships

Snow crab recruitment in the EBS was highly variable, cyclic, and positively correlated among alternative indices at varying lags. Time series of natural logarithm-transformed recruitment were stationary but significant autocorrelation was observed in all indices. Cyclical fluctuations were observed in all recruitment indices and suggested a periodicity of 5-6 yr (Fig. 3.5a). This cycle was particularly clear following the trough in recruit abundance in 1996 or 1997 depending

on the index evaluated (Fig. 3.5a). Cross-correlation among indices was positive and significant (adjusted for autocorrelation by *N**) with peak correlation observed at varying time lags (Fig. 3.5b). Instar vii abundance was significantly correlated with instar viii abundance at lags of 0 (r = 0.68) and +1 (r = 0.77) yr (Fig. 3.5b). Instar vii and instar viii recruitment were significantly correlated with recruitment estimated from the length-based assessment model, at lags of 0 (r = 0.69) and -1 (r = 0.54) year (Fig. 3.5b), respectively. Instar vii abundance was identified as the preferred index for stock-recruitment analyses due to the strong correlation with model-estimated recruitment and status as a robust leading indicator of instar viii abundance.

The functional form of best-fit stock-recruit relationships varied with the time lag from the indexed reproductive stage (fertilization or oviposition) to recruitment (Table 3.3). All models indicated improved model fit ($\Delta AIC_c \geq 2$) with first-order autocorrelation of the residuals. Rho *(ρ)*, the first-order autocorrelation parameter, was estimated for all fits to the data. The density-independent model, indicating increasing average recruitment with increasing reproductive potential, was identified as the best fit model for all indices of reproductive potential aggregated for the stock at lag of 4 yr (Table 3.3, Fig. 3.6). In contrast, mean recruitment (e.g. constant recruitment over all values of spawning stock) was the best fit and only competitive model for comparisons between the abundance of adult male, mature male, mature female, and summer egg production and recruitment at a lag of 5 yr (Table 3.3, Fig. 3.6). A preferred index of reproductive potential robustly related to variability in recruitment within each time lag was not clearly identified. Beverton-Holt, and in some cases Ricker, relationships were identified as plausible (competitive for adult males) relationships at both lags for mature indices and summer egg production. However, in most cases parameter estimates indicated a functional form of the model very similar to that of the density-independent or mean recruitment fits (Table 3.3, e.g. Fig. 3.6a). Among relationships at lag 4 the density-independent fit of both mature female and summer EPI had the lowest observed AIC_c and RMSE values by small margins indicating a trend towards better fit of indices of female reproductive potential to the recruitment data versus indices of male reproductive potential.

Stock-recruit relationships based on egg production in the middle domain and southern middle domain differed from those of aggregate indices. High recruitment values at low to intermediate levels of reproductive potential observed in these comparisons contrasted with the relative lack

of coherence at lag 5 or the increasing average recruitment and reproductive potential at lag of 4 yr for aggregate indices (Fig. 3.6). Mean recruitment was the only competitive model at a lag of 4 yr (Table 3.3). Mean recruitment and two-parameter models with forms closely approximating the mean were competitive at lag 5 (Table 3.3, Fig. 3.6).

Temporal patterns were evident in the autocorrelation-adjusted residuals of stock-recruitment relationship (anomalies from mean recruitment for comparison for which mean recruitment was identified as the best model) (Fig. 3.6a) and indices of stock productivity estimated as the natural logarithm of recruits per unit reproductive potential (ln R/S) for EBS snow crab (Fig. 3.8). Two anomalously high recruitment events during the late 1980s, followed by a strong negative anomaly in the early 1990s were evident in the residuals of the stock-recruitment relationships (Fig. 3.7a & 3.7c), with quasi-cyclical variability with periods of positive and negative anomalies thereafter through the 2007/09 brood year. Similarly, indices of stock productivity suggested a period of very low followed by high stock reproductive potential coincident with high stock productivity from the mid to late 1980s followed by a historic minimum in recruits per spawner for brood years in the early to mid-1990s depending on the index considered (Fig. 3.8). This minimum was followed by higher levels of productivity and which did not qualitatively correspond to variation in reproductive potential (Fig. 3.8).

3.4 Discussion

Biennial reproduction has been confirmed for females associated with cold water in the EBS (< ~0-1°C) (Armstrong et al., 2008), but a lack of comprehensive empirical data motivated us to develop methods to model the impact of biennial reproduction on reproductive potential of female snow crab in the EBS. Simple stage-based models fitted to the female abundance data provided reasonable parameter estimates for survival and stage transition (Table 1), but application of these parameters to the observed data at varying threshold temperatures revealed strong bias in the inference of year-to-year variation in the proportion biennial based on long-term averages (Fig. 3.3b).

Biased values evident in the inference of the proportion biennial in this study may have arisen from uncertainty in the underlying data or model assumptions. First, stage-specific abundance estimates were likely impacted by the fixed spatial density of survey sampling density relative to

the spatial dynamics of mature females which vary with ontogeny. The spatial distribution of mature females becomes increasingly aggregated from newshell to very oldshell in the EBS (Ernst et al., 2005, Murphy et al., 2010, Zheng et al., 2001). For example, the stage-based model estimate of very old shell abundance was much lower than the area-swept estimate in 1989. Greater than one-half of this anomalously high estimate was caught at a single survey station. We investigated use of the geometric mean to reduce the influence of anomalous tows on survey abudance indices (McConnaughey and Conquest, 1993), but this approach provided very similar results (J. Webb, unpublished data). Second, the stage-based modeling approach assumes a closed population. Model estimated abundance of oldshell females in this study was markedly lower than that observed in the survey in the early 1990s, coincident with the immigration of a large cohort of oldshell females into the survey area from the north in the late 1980s that persisted into the early 1990s (Ernst et al., 2005, 2012). Third, high variability in the ratio of newshell to oldshell females in the following year could be the result of high temporal variability or stage-specific natural mortality rates (Vetter 1988), inaccuracies in the assignment of shell condition index (e.g. Fonseca et al. 2008), or as a consequence of expansion of geographic frame of the trawl survey during the 1980s (Daly et al., 2014).

Further investigation of the prevalence of biennial reproduction in the EBS snow crab stock via new or existing field studies and the analysis of existing datasets is warranted given the limited success of our model-based approach. In Nova Scotia, Canada, egg clutch color data were collected during trawl surveys from early spring through late winter and biennially reproducing females were successfully differentiated from annually reproducing females using the variability in the color which changed seasonally with embryo development stage (Kuhn and Choi, 2011). Unfortunately, similar efforts were unsuccessful for the EBS snow crab (Armstrong et al., 2008), likely due to the timing (summer) and short duration (~2 mo) of the EBS trawl survey relative to Nova Scotia. Identifying females on a biennial cycle in the EBS would require sampling in areas both inside and outside of the outer domain proximate to the spatial extent of the cold pool in the prior year. Further analysis of the results of a comprehensive seasonal study of female maturity, reproductive potential and biennial reproduction in contrasting cold and warm locations in the EBS (see Pengilly et al., 2014) could also provide insight useful for refining modeling approaches.

Estimates of average mature female survival derived from the stage-based models in this study (Table 1) were similar to those estimated in other studies. A survival rate of 57-59% was estimated for mature female EBS snow crab (Zheng, 2003). Similarly, average annual survival rate of mature female snow crab in the Gulf of St. Lawrence, Canada, was estimated at 45% or 52% (under varying assumptions) using trawl survey time series of abundance of newshell and oldshell females and cyclic variability in female size (Drouineau et al., 2013) and at 57% by tag-recapture for a cohort of female snow crab within a area closed to fishing in Japan (Yamasaki et al., 2001). These estimates of survival are notably lower than those currently applied to mature females in the length-based stock assessment model for EBS snow crab (S=0.79, M=0.23, Turnock and Rugolo, 2013). Longevity following the terminal molt to maturity inferred from average survival rates (5% to 7 yr and 1% to 9 yr) is also comparable with published estimates (Ernst et al., 2005; Kon et al., 2010).

Consistent with current knowledge of life history and spatial dynamics, a high proportion of the newshell mature female snow crab in the EBS are associated with cold water but association decreases markedly for oldshell and very oldshell females (Fig. 3.3a). Association of early benthic stages and immature snow crab with cold water is likely a result of early life history processes. Direct information on settlement (e.g. behavior and potential settlement cues for post-larvae) is not available for the EBS, but field studies in Japan suggest that post-larval snow crab have substantial ability to modify their position in the water column and actively search for optimal settlement habitats with temperature as a possible cue (Kon et al., 2003; Kon and Sinoda, 1992). Early benthic stage snow crab are found almost exclusively at temperatures of less than about 2° C in the EBS (Parada et al., 2010). These life stages are stenothermic and exhibit active preference for waters with near-bottom temperature of ~0-2°C (Dionne et al., 2003). Juvenile snow crab are thought to be sedentary such that female snow crab grow from the early benthic stages to maturity in the cold waters of the middle domain (Ernst et al., 2005) where biennial reproduction is induced when embryos are exposed to cold ambient temperatures (<~1°C) early in embryo development (Armstrong et al., 2008; Moriyasu and Lanteigne, 1998; Webb et al., 2007). Following the terminal molt to maturity females move into the warmer (> ~2 °C) waters of the outer domain, hypothetically oriented by gradients in bottom temperature (Ernst et al., 2005, Parada et al., 2010). Under this scenario, oldshell females arriving in the outer domain would be observed brooding clutches in the intermediate year of the biennial reproductive cycle

during the summer trawl survey. However, induction of subsequent biennial reproductive cycles would be unlikely, leading to low rates of biennial reproduction among very oldshell females.

Independent of biennial reproduction, integrating size-frequency, shell condition, and clutch fullness index information into estimates of annual egg production resulted in lower estimated reproductive potential for EBS snow crab compared to simpler indices (Fig. 3.4b). Importantly, interannual variability in the magnitude of the difference in egg production (CV = 0.76) between simpler and more refined indices egg production suggested that demographic factors are not a simple scalar effect. Strong divergence between simple and more refined indices of egg production was observed from the early 1990s forward. Shifts in mature female demography, size and shell condition composition, may be the cause of this divergence based on two lines of evidence. First, a reduction in the mean size of newshell mature females was observed across regions of the EBS shelf (-5-10%) (Orensanz et al., 2007), resulting in a potential 20-30% decrease in individual fecundity depending on the CFI category (Webb, Chapter 2 this dissertation). Second, higher proportional abundance of old and very oldshell females with lower than average CFI (Ernst et al., 2012; Webb, Chapter 2 this dissertation) in both the 1990s (Zheng et al., 2001) and 2000s (Webb, unpublished data) may have reduced estimated egg production relative to earlier in the time series. In addition, estimates of egg production may have also been impacted by methodological factors. A standardized reference for scoring CFI was implemented on the EBS trawl survey in the early 1990s resulting in higher proportions of females, principally newshell, being scored with lower CFI than in previous years (Orensanz et al., 2005). Although this change in protocol influenced CFI distributions, CFI time series prior to implementation are still considered useful for estimation of egg production due to the interpretable relationship between indices of mean CFI and mean shell condition (Ernst et al., 2012).

In this study, we used an area-swept abundance index of juvenile EBS snow crab corresponding to instar vii, 25-33 mm CW, and likely ~3 - 4 yr post-settlement (Kilada et al., 2012, Sainte-Marie et al., 1995) compared to a broader size range of 25 to 40 mm CW juveniles used in previous studies of snow crab stock and recruitment dynamics (Zheng and Kruse, 2003; Zheng and Kruse, 2006). Analysis of size-frequency distributions of juvenile EBS snow crab in cod stomachs and in trawl survey data indicated that instar vii juveniles were reliably observed in the trawl survey (Ernst et al., 2012). Cross-correlations (Fig. 3b) and patterns of lag and overlap

between peaks and troughs of abundance of instar vii and instar viii juveniles (Fig. 3a) clearly demonstrated that instar vii abundance was a leading indicator of the abundance of instar viii juveniles. Instar vii abundance was also robustly correlated with the length-based model index of recruitment reported in annual stock assessments (Turnock and Rugolo, 2013), earlier versions of which have been used in other recent studies of snow crab recruitment variability (Hinckley et al., 2010; Marcello et al., 2012; Szuwalski and Punt, 2013). Interpretation of instar vii abundance and other recruitment indices as reliable indices of year-class strength may be affected by several factors including temperature-driven differences in molt frequency within the first few years post-settlement (Burmeister and Sainte-Marie, 2010; Ernst et al., 2012; Orensanz et al., 2007), presence of several age-classes in the size range of instar vii (Kilada et al., 2012), and spatiotemporal variability in the intensity of predation of juvenile snow crab by Pacific Cod at sizes smaller than instar vii (Burgos et al., 2013). Interpretation of indices of recruitment estimated from length-based assessments also have limitations as the model is re-estimated annually with updated input data; revision of historical data; and structural changes to the model or assumptions regarding survey selectivity, growth, and natural mortality.

Patterns observed in stock-recruit relationships and stock productivity, indexed by ln R/S, in this study provide important insight into factors affecting stock renewal processes for EBS snow crab. The positive linear relationship between recruitment and reproductive potential observed in this study, clearest in comparisons using summer EPI versus other indices (Table 3.3, Fig. 3.6) implies that variability in reproductive potential has a discernible role in stock dynamics. Four brood years in the late 1980s were the primary drivers of this relationship. The choice of lag from oviposition/fertilization to recruitment was key to observation of this pattern. We consider a four year lag to be a reasonable assumption given current knowledge of the life history schedule of female snow crab in the EBS (Ernst et al., 2012) and eastern Canada (Comeau et al., 1998), but the prevalence of biennial reproduction and hypothesized temperature-driven latitudinal variation in size-at-age (Ernst et al., 2012, Orensanz et al., 2007) would contribute to uncertainty in determining the correct lag. Assumptions regarding the lag from brood year to recruitment are also critical for analysis of the effects of environmental variability on recruitment dynamics and the of lag of 4 yr used in this study differs from the lag of 5 yr which has been assumed other studies and the stock assessment (Turnock and Rugolo, 2013). Strong autocorrelation in all stock-recruit comparisons, consistent with prior studies (Zheng and Kruse, 2003, 2006), could be

the result of the processes contributing to uncertainty in determining the correct lag and/or external drivers affecting recruitment (e.g. Szuwalski and Punt, 2013). Empirical age composition data (e.g. Kilada et al., 2012) for instar vii and viii in the EBS would be useful to investigate uncertainly in the assumed lag from reproduction to recruitment due to biennial reproduction and growth rate variability. Refining indices of reproductive potential to reflect the contribution of females in the middle domain, hypothesized to play a key role in stock renewal (Parada et al. 2010), did not result in a comprehensible relationship with aggregate recruitment in our analysis. However, further refining these analyses to more realistically reflect spatiotemporal variation in oceanographic connectivity in the region of snow crab distribution in the EBS (Danielson et al., 2012, Richar et al., in press) could provide further insight into their potential impact on recruitment success.

High stock productivity appears to have sustained recruitment through a historic minima in reproductive potential in the mid 1980s followed by a dramatic decline in stock productivity and subsequent minima in the early 1990s (Fig 3.8). This decline in stock productivity could be the result of climatic regime shifts indexed by the Pacific Decadal Oscillation in 1989 (Szuwalski and Punt 2013), reduced abundance of female EBS snow crab in the southern portion of the distribution (Ernst et al., 2012), or the result of shifts in the location and intensity of Pacific cod predation on juvenile snow crab (Burgos et al., 2014). These patterns suggest that, under favorable conditions in the mid to late 1980s, high levels of reproductive potential resulted in the formation of the strong year-classes. On the other hand, high levels of recruitment failed to materialize from high levels of estimated egg production in the early 1990s, perhaps due to unfavorable conditions for larval and juvenile survival.

Similar to other commercially important crustaceans, sex-specific harvest, high recruitment variability, complex life histories, and the presumed strong influence of density-independent factors on population dynamics (Caputi 1993, Wahle 2003) have challenged the development of reliable stock-recruit relationships for EBS snow crab (Zheng and Kruse 2003, 2006). Despite concerted efforts, the refined indices of female reproductive potential developed in this study did not reveal a robust, definitive stock-recruit relationship, which is required for a EBS snow crab to be managed under the MSY-based reference point framework (Tier 1) defined in Amendment 24 to the fishery management plan for Bering Sea and Aleutian crab stocks (NPFMC, 2011). The

sex-specific nature of the EBS snow crab fishery also complicates the use of per recruit analyses, which presume reductions in female reproductive potential are due to fishing mortality for estimation of MSY reference points. In the case of female EBS snow crab, the known impact of fishing (e.g. bycatch mortality) is small (Turnock and Rugolo, 2013), but female mortality may also be mediated by fishery impacts on sex ratio during the mating period. Female mortality may increase with intense male-male competitition for mating opportunties when sex ratios are highly male-biased or females may be killed for attempting coercion of additional sperm reserves from males under highly female-biased conditions (Rondeau and Sainte-Marie 2001, Sainte-Marie et al., 2008). There is limited information available to retrospectively evaluate whether sperm limitation, another potential mechanism for fishery-induced reductions in female reproductive potential (Sainte-Marie et al., 2008), may have occurred for EBS snow crab. Female clutch size fluctuates in relation to female demography, indexed by shell condition (Ernst et al., 2012; Webb et al.,Chapter 2 this dissertation) and extensive monitoring of sperm reserves and egg viability of EBS snow crab in recent years has not detected sperm limitation (Slater et al., 2010, L. Stichert, unpublished data).

The results of this and other recent studies suggest three corollary points important to fishery management of EBS snow crab. First, extensive recent investigations have resulted in improved understanding of variability in reproductive potential, distribution and movement, life-history schedule, metapopulation structure and biophysical connectivity, and other factors impacting stage-specific survival. All of these advance the idea that EBS snow crab stock renewal dynamics are a complex, yet comprehensible, product of these processes. If indices capturing key elements of these factors can be developed, further quantitative analyses could advance knowledge of the role of viable egg production on stock productivity, thus providing a key ingredient to efforts to identify the effective spawning stock from which to base biological reference points for fishery management. Investigation of potential stock-recruit relationships could also be improved by development of an empirical data to describe spatiotemporal variation in biennial reproduction and age-age composition (Kilada et al., 2012). Finally, virtually all of the recent progress on EBS snow crab life history, spatial dynamics, and population dynamics is based on females. Progress has been made on use of chela morphometry to determine male maturity in stock assessment (since 2004). However, further understanding of male dynamics, such as life-history schedule, spatial dynamics, harvest effects on demography/distribution, and

sex ratio at mating (summarized in Pengilly et al., 2014), will be necessary for a more complete understanding of the dynamic conditions under which male-only harvest impacts stock fertilized egg production.

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Table 3.1. Parameter estimates from the stage-based model by time period fitted and profile confidence intervals (95%) for annual survival fraction ($\hat{S}$) and the annual proportion of oldshell females remaining at the oldshell stage ($\hat{P}_o$) for mature female snow crab in the eastern Bering Sea. Parameters are estimated from the stage-based model of abundance by shell condition class fit to portions of the time-series based on changes to the sampling gear, survey coverage, and patterns of variation in abundance by stage.

Time Period	$\hat{S}$	$\hat{P}_o$
1978-2012	0.50 (0.43, 0.57)	0.58 (0.34, 0.74)
1982-2012	0.53 (0.46, 0.61)	0.61 (0.37, 0.77)
1989-2012	0.60 (0.52, 0.68)	0.70 (0.49, 0.83)
1991-1999	0.49 (0.36, 0.62)	0.72 (0.39, 0.89)

Table 3.2. Association among indices of reproductive potential and egg production indicated by Pearson's product-moment correlation coefficient (*r*) and 95% confidence intervals (indicated in parentheses) between first-differenced time-series of egg production, summer (SUM), middle domain (MD), and southern middle domain (SMD), and area-swept abundance of adult male (AM), mature male (MM), and mature females (MF) for eastern Bering Sea snow crab. Asterisks (*) indicate a statistically significant correlation at the corresponding threshold.

	AM	MM	MF	SUM	MD	SMD
AM		0.92 (0.85-0.96)	0.66 (0.42-0.82)	0.70 (0.47-0.84)	0.50 (0.19-0.72)	0.30 (-0.04-0.58)
MM	***		0.63 (0.38-0.80)	0.68 (0.44-0.83)	0.47 (0.15-0.70)	0.31 (-0.02-0.59)
MF	***	***		0.97 (0.93-0.98)	0.40 (0.44-0.82)	0.33 (-0.01-0.60)
SUM EPI	***	***	***		0.76 (0.57-0.87)	0.44 (0.12-0.68)
MD	*	**	*	**		0.91 (0.83-0.96)
SMD	ns	ns	ns	**	**	

*** - $p<0.001$, ** - $p<0.01$, * - $p<0.05$, ns- not significant

Table 3.3. Parameter estimates for stock-recruitment models assuming lags of 4 and 5 yr. from the indexed stage (oviposition or fertilization) to recruitment. Mean recruitment, density-independent (DI, linear) and two-parameter stock-recruitment relationships (Ricker and Beverton-Holt) with auto-correlated error structure were fit to each relationship. Fitted models were evaluated by ΔAIC_c and RMSE. See text for definitions of indices of reproductive potential (RP). Fitted relationships of competitive models ($\Delta AIC_c<2$) are shown in Fig. 6.

Index RP	Adult Male							
Lag (yr)			4				5	
Model	Mean	DI	Ricker	Beverton-Holt	Mean	DI	Ricker	Beverton-Holt
α		0.14	0.14	11068		0.33	0.67	10.00
β	8.51		0.00	80397	10.00		0.02	0.00
ρ	0.72	0.78	0.78	0.78	0.73	0.75	0.72	0.73
AIC_c	30.18	24.67	29.74	26.67	24.87	33.99	27.38	27.35
ΔAIC_c	5.51	0.00	5.07	2.00	0.00	9.12	2.51	2.48
RMSE	18.58	17.85	17.85	17.85	18.76	19.41	18.53	18.76
Index RP	Mature Male							
Lag (yr)			4				5	
Model	Mean	DI	Ricker	Beverton-Holt	Mean	DI	Ricker	Beverton-Holt
α		0.73	0.73	17117		0.87	3.09	10.18
β	8.51		0.00	23654	10.00		0.10	0.18
ρ	0.72	0.77	0.77	0.77	0.73	0.76	0.72	0.73
AIC_c	30.20	27.32	29.77	29.77	24.87	28.76	27.51	27.35
ΔAIC_c	2.88	0.00	2.45	2.45	0.00	3.89	2.64	2.48
RMSE	18.58	18.07	18.07	18.07	18.75	19.27	18.53	18.75
Index RP	Mature Female							
Lag (yr)			4				5	
Model	Mean	DI	Ricker	Beverton-Holt	Mean	DI	Ricker	Beverton-Holt
α		0.59	0.86	23739		0.71	2.02	10.15
β	8.51		0.02	40209	10.00		0.06	0.14
ρ	0.72	0.81	0.72	0.81	0.73	0.77	0.74	0.73
AIC_c	30.18	23.38	25.82	25.83	24.87	33.40	30.63	27.33
ΔAIC_c	6.80	0.00	2.44	2.45	0.00	8.53	5.76	2.46
RMSE	18.58	17.10	16.98	17.04	18.75	20.06	18.76	19.09
Index RP	Summer EPI							
Lag (yr)			4				5	
Model	Mean	DI	Ricker	Beverton-Holt	Mean	DI	Ricker	Beverton-Holt
α		0.24	0.24	27600		0.29	0.76	10.05
β	8.51		0.00	93020	10.00		0.02	0.11
ρ	0.72	0.81	0.81	0.81	0.73	0.80	0.74	0.73
AIC_c	30.20	22.73	25.19	25.29	24.89	33.57	31.69	27.39
ΔAIC_c	7.47	0.00	2.46	2.56	0.00	8.68	6.80	2.50
RMSE	18.59	16.60	16.60	16.25	18.76	19.79	19.23	18.75
Index RP	Middle Domain							
Lag (yr)			4				5	
Model	Mean	DI	Ricker	Beverton-Holt	Mean	DI	Ricker	Beverton-Holt
α		0.54	0.16	12.00		0.64	1.21	13.86
β	8.51		0.00	4.64	10.00		0.03	4.35
ρ	0.72	0.76	0.83	0.71	0.73	0.77	0.76	0.75
AIC_c	30.21	32.61	33.66	31.43	24.86	28.07	27.01	25.33
ΔAIC_c	0.00	2.40	3.45	1.22	0.00	3.21	2.15	0.47
RMSE	18.58	19.34	17.93	18.24	18.76	19.20	19.00	18.59
Index RP	Southern Middle Domain							
Lag (yr)			4				5	
Model	Mean	DI	Ricker	Beverton-Holt	Mean	DI	Ricker	Beverton-Holt
α		1.18	1.91	11.53		1.48	2.98	12.12
β	8.51		0.03	1.04	10.00		0.05	0.53
ρ	0.72	0.82	0.75	0.70	0.73	0.80	0.76	0.75
AICc	30.19	35.82	36.61	32.07	24.87	35.04	31.85	24.29
ΔAICc	0.00	5.63	6.42	1.88	0.58	10.75	7.56	0.00
RMSE	18.58	21.70	17.16	18.00	18.76	23.67	19.71	18.61

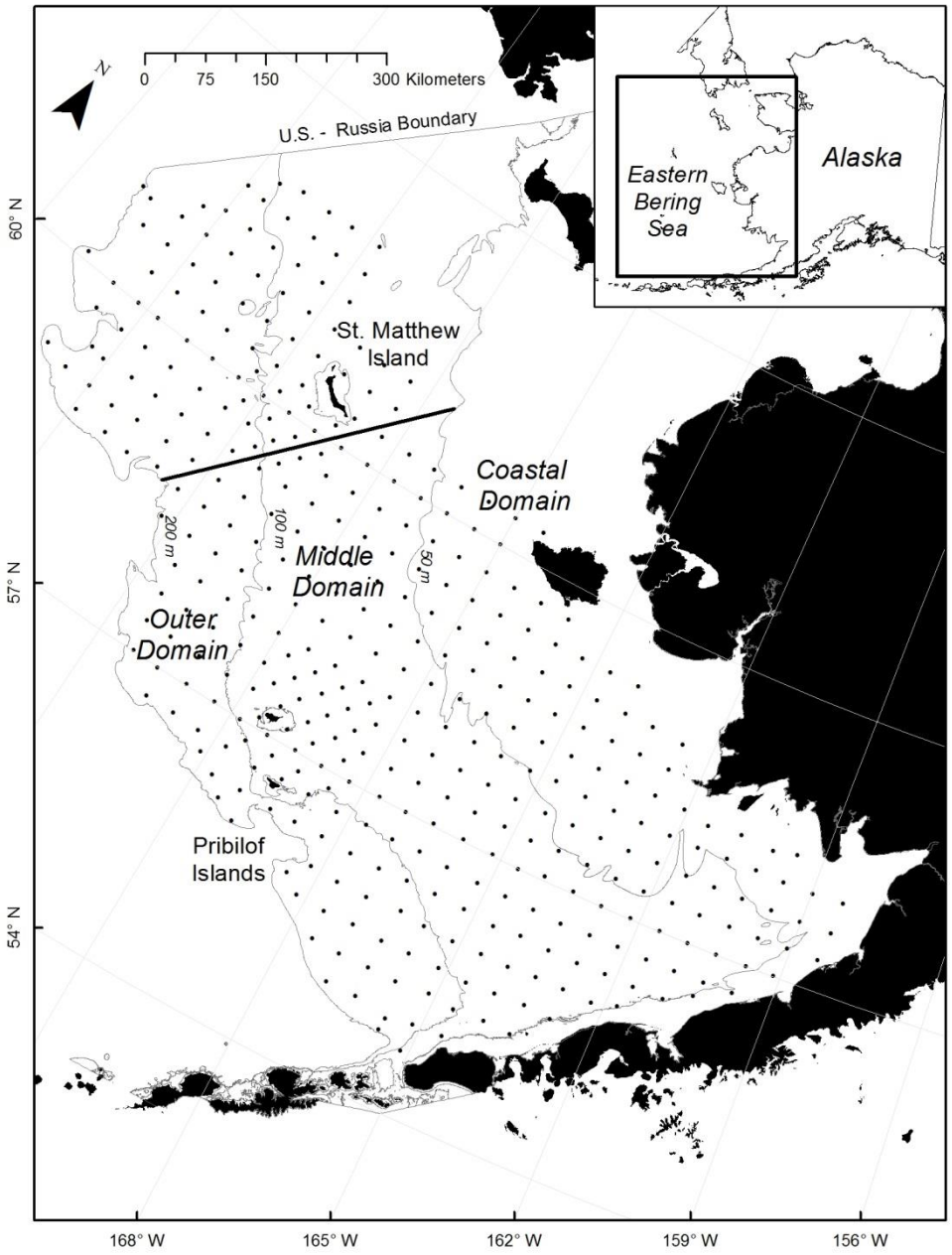


Figure 3.1. Map of study area in the eastern Bering Sea with oceanographic domains delineated by bathymetry (lines) and station locations (filled circles) of the bottom trawl survey conducted annually by the National Marine Fisheries Service. The solid straight line across the middle and outer domain indicates the boundary used divide the northern and southern portion of the middle domain for estimation of egg production indices

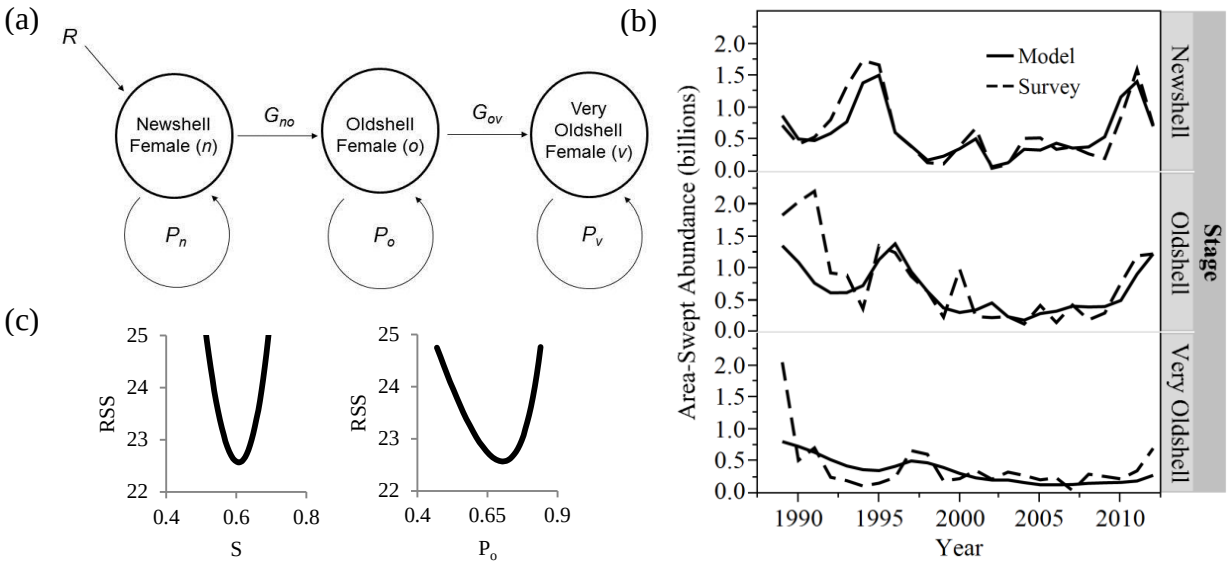


Figure 3.2. Results of stage based model of mature female abundance for eastern Bering Sea snow crab: (a) model structure (see text for description of parameters), (b) trawl survey area-swept and stage-based model (using $\hat{S}$ = 0.60 and $\hat{P}_o$ = 0.70) indices of mature female abundance by shell condition class, and (c) likelihood profiles of annual survival fraction ($\hat{S}$) and the annual probability of females staying in the oldshell shell condition class ($\hat{P}_o$) for mature female snow crab in the eastern Bering Sea from 1989 to 2012.

(a)

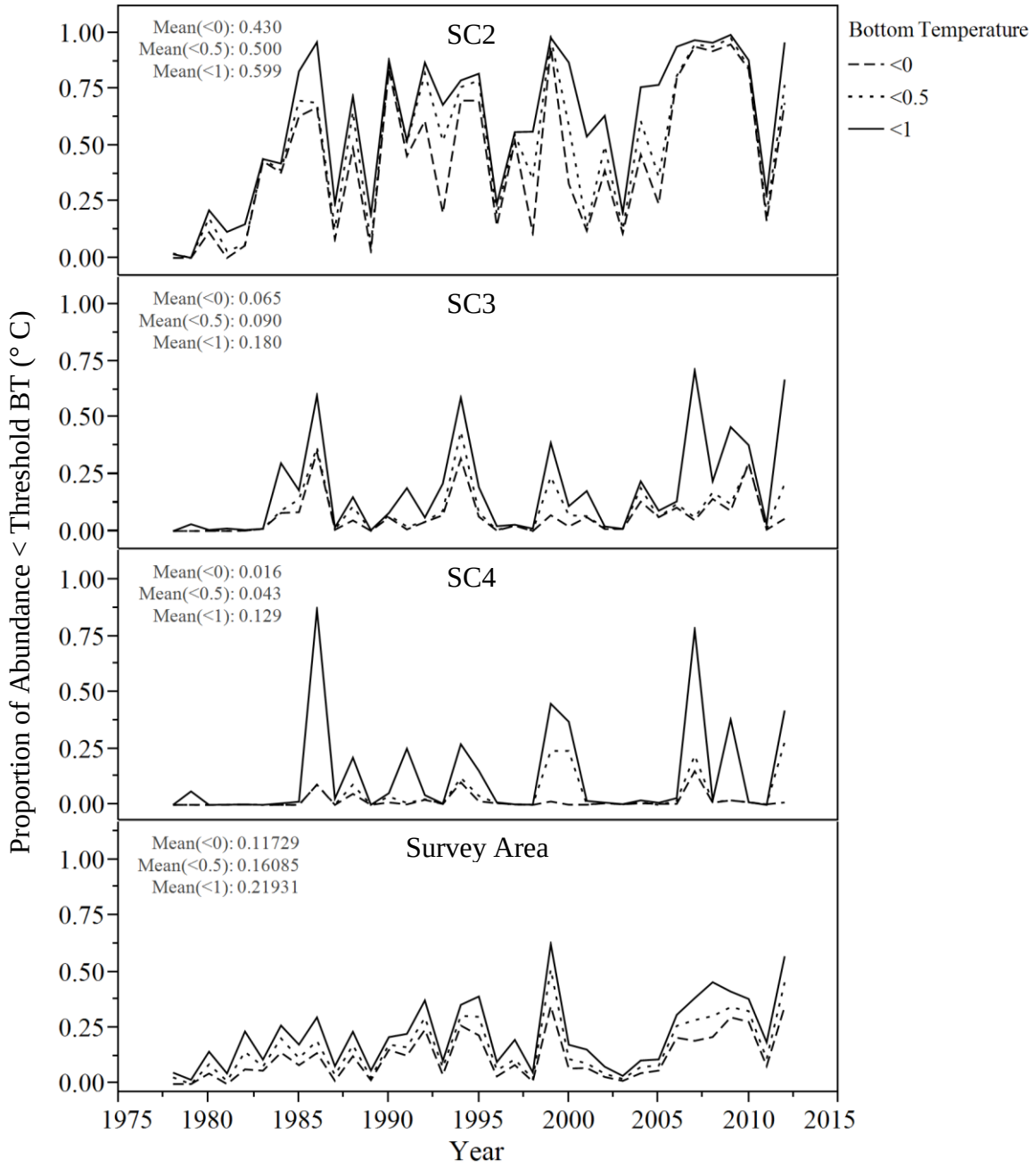


Figure 3.3. Association with cold bottom water and inferred proportion biennial for eastern Bering Sea snow crab from 1978 to 2012. (a) The proportion of the area-swept abundance estimate of newshell (SC2), oldshell (SC3), and very oldshell (SC4) female snow crab and proportion of the total survey area with bottom temperatures (BT) below 0°, 0.5, and 1° C and (b) proportion of oldshell and very oldshell female snow crab inferred to be reproducing biennially (e.g. not undergoing oviposition and brooding of an egg clutch in the intermediate stages of development) inferred by the distribution in bottom water below the threshold temperature and survival and stage-transition parameters estimated from stage-based model fits. Proportions > 1 indicate estimation bias.

(b)

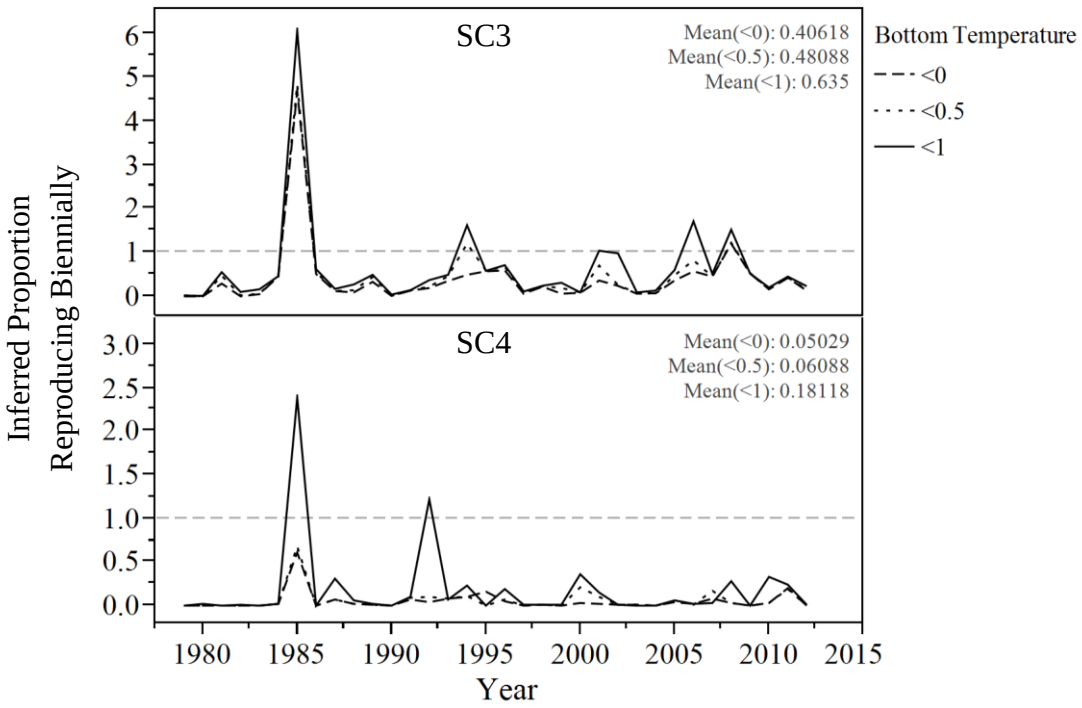


Figure 3.3. continued

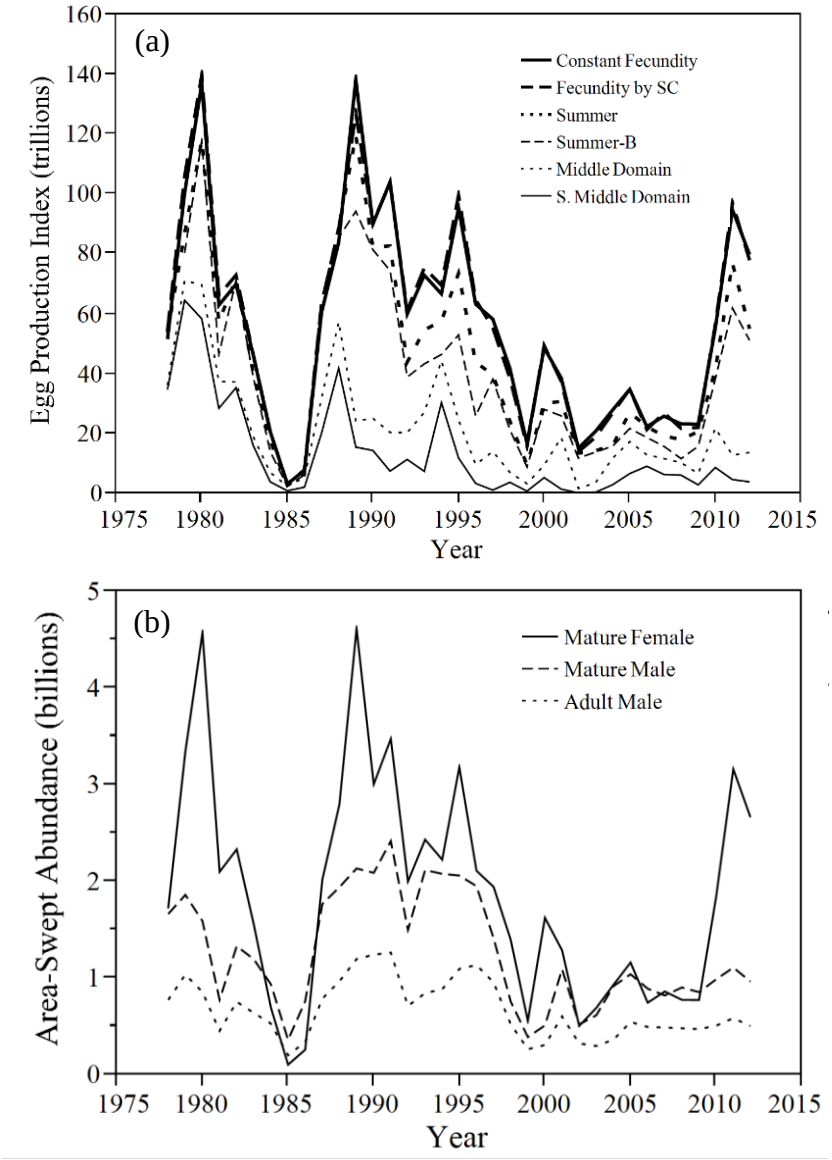


Figure 3.4. Snow crab egg production and mature abundance in the eastern Bering Sea (EBS) from 1978 to 2012. Egg production indices (a) were calculated under assumptions of constant fecundity for all females (constant fecundity), variation in fecundity by female shell condition (fecundity by SC), size and clutch fullness index (Summer), summer decremented for biennial reproduction (Summer-B), summer including only females in the middle domain of the EBS (Middle Domain), and summer including only females in the southern middle domain (see Fig. 1) of the EBS (S. Middle Domain). Area-swept indices (b) were obtained from the summer bottom trawl survey of mature female, mature male, and adult male abundance (see text for definitions of maturity) for eastern Bering Sea snow crab from 1978 to 2012.

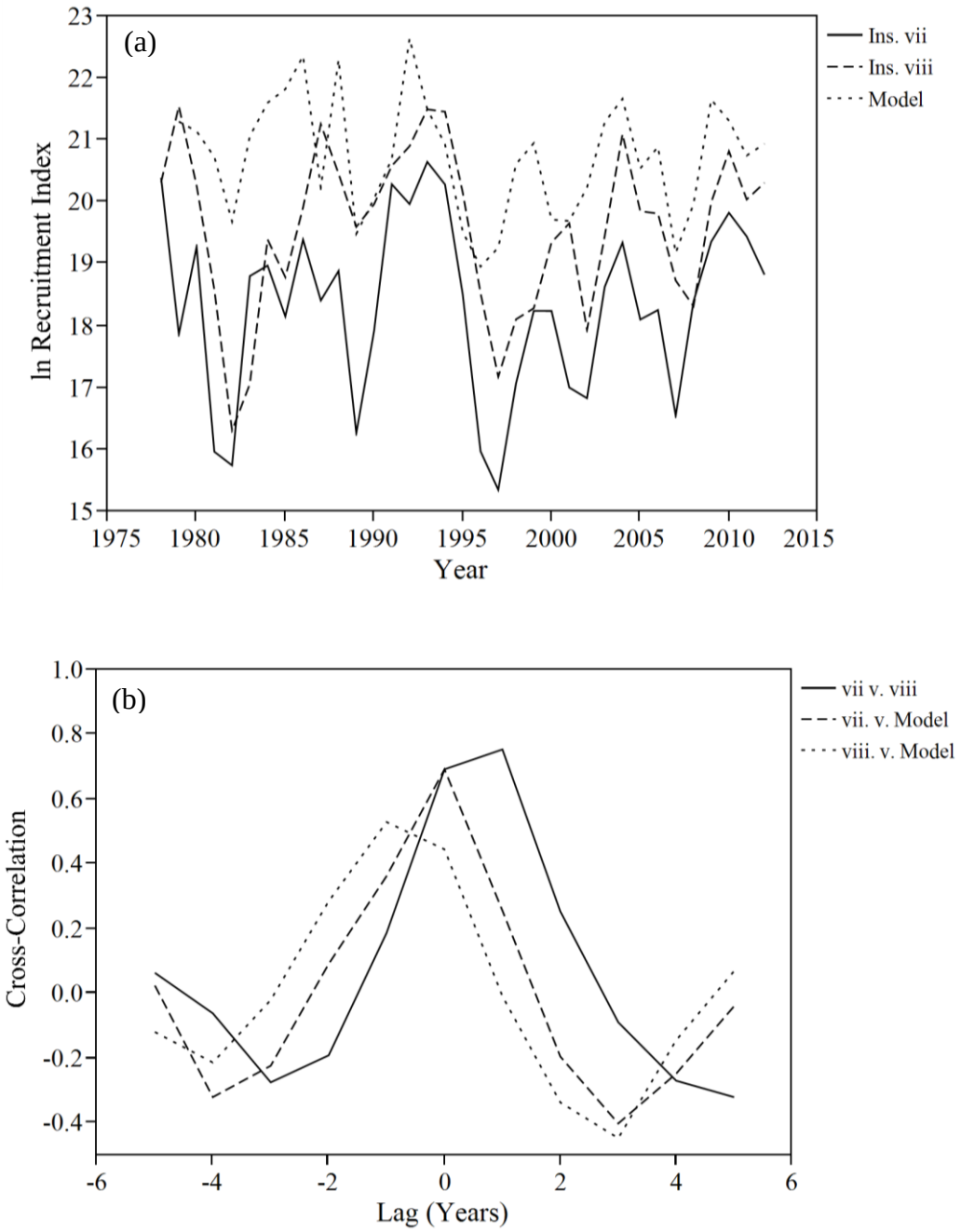


Figure 3.5. Snow crab recruitment indices and their cross-correlations for the eastern Bering Sea(a) Recruitment indices over 1978 to 2012 based on the natural logarithm of area-swept abundance of instar vii (25-33 mm CW), instar viii (34-43 mm CW), and juvenile recruits (25-40 mm CW) estimated from a stock assessment model; and (b) cross-correlations among these indices. Cross-correlations, corrected for autocorrelation using effective sample size (N^*), were significant ($p<0.05$) at lags of -2 and -1 years for instar vii vs. model, at -1 for instar viii vs. model, and lags 0 and 1 for instar vii vs. instar viii.

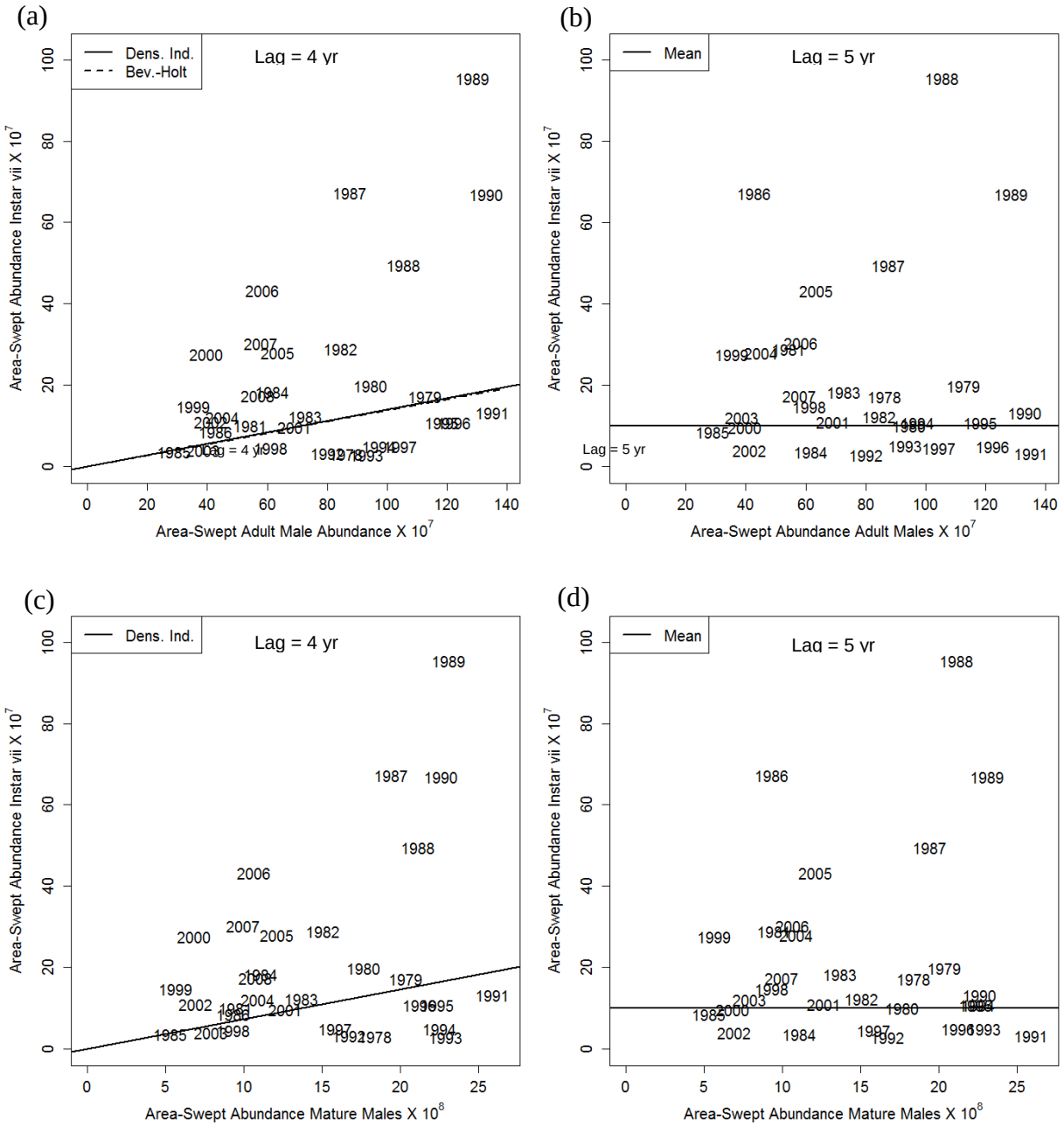


Figure 3.6. Stock-recruitment relationships for snow crab in the eastern Bering Sea identified as competitive ($\Delta AIC_c<2$) between indices of reproductive potential (x-axis) and recruitment (y-axis) from mean recruitment to two-parameter stock-recruitment relationships (Ricker, Beverton-Holt) with auto-correlated error structure. Years within each plot are the year of fertilization or brood oviposition for indices of male abundance, female abundance, and various egg production indices. The assumed time lag (yr) between the index of reproductive potential and recruitment is indicated within each panel.

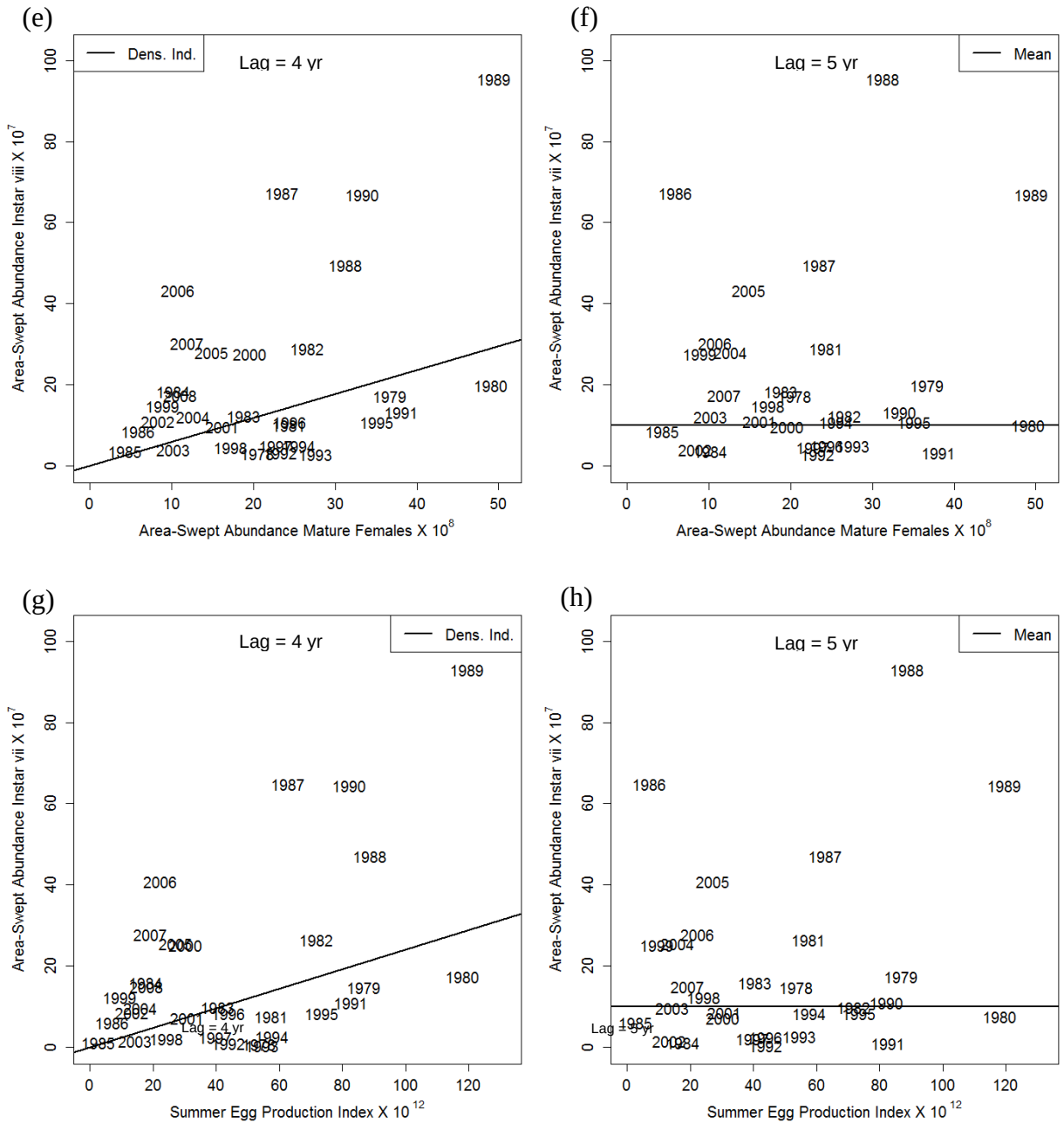
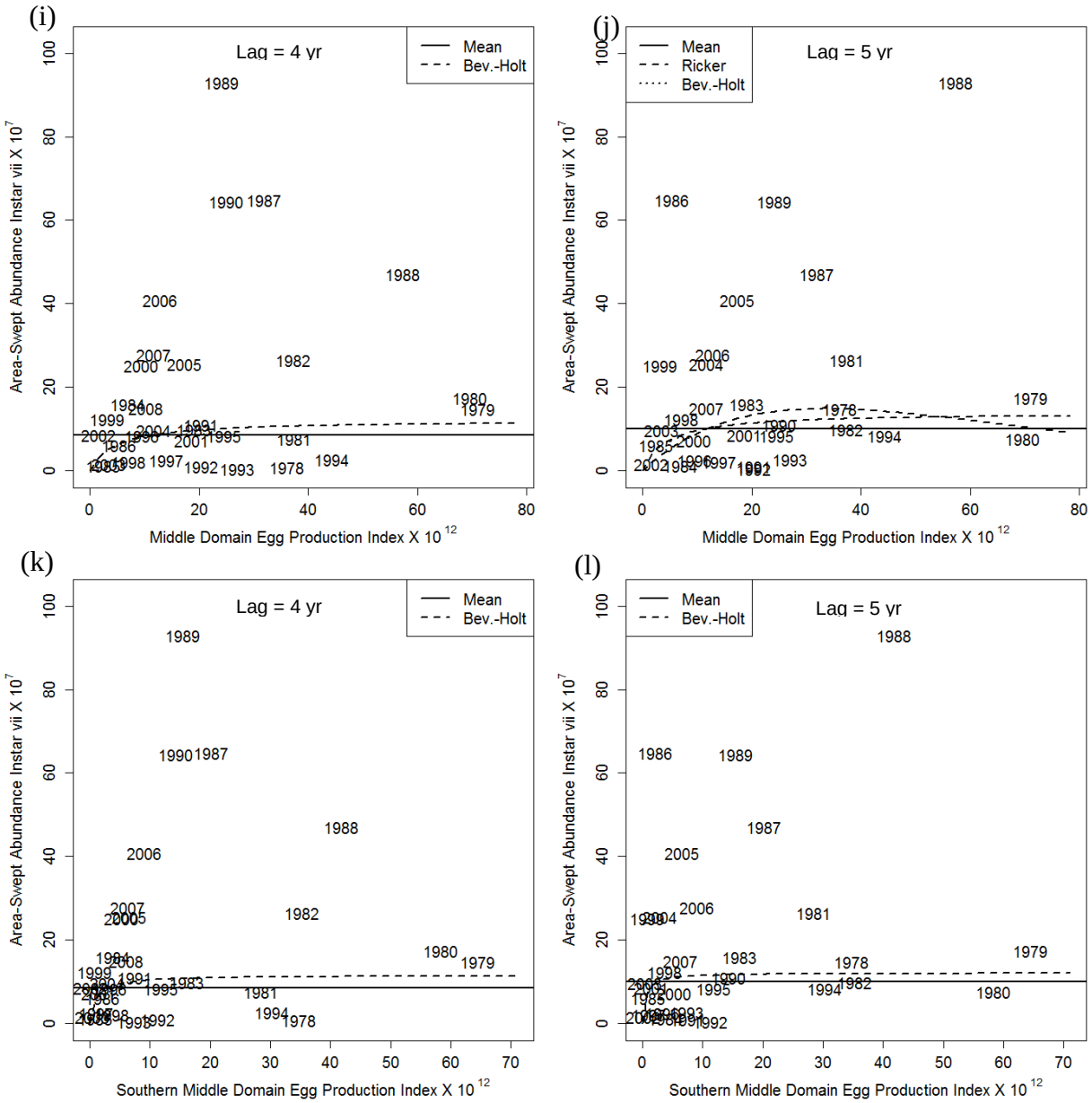


Figure 3.6. Continued



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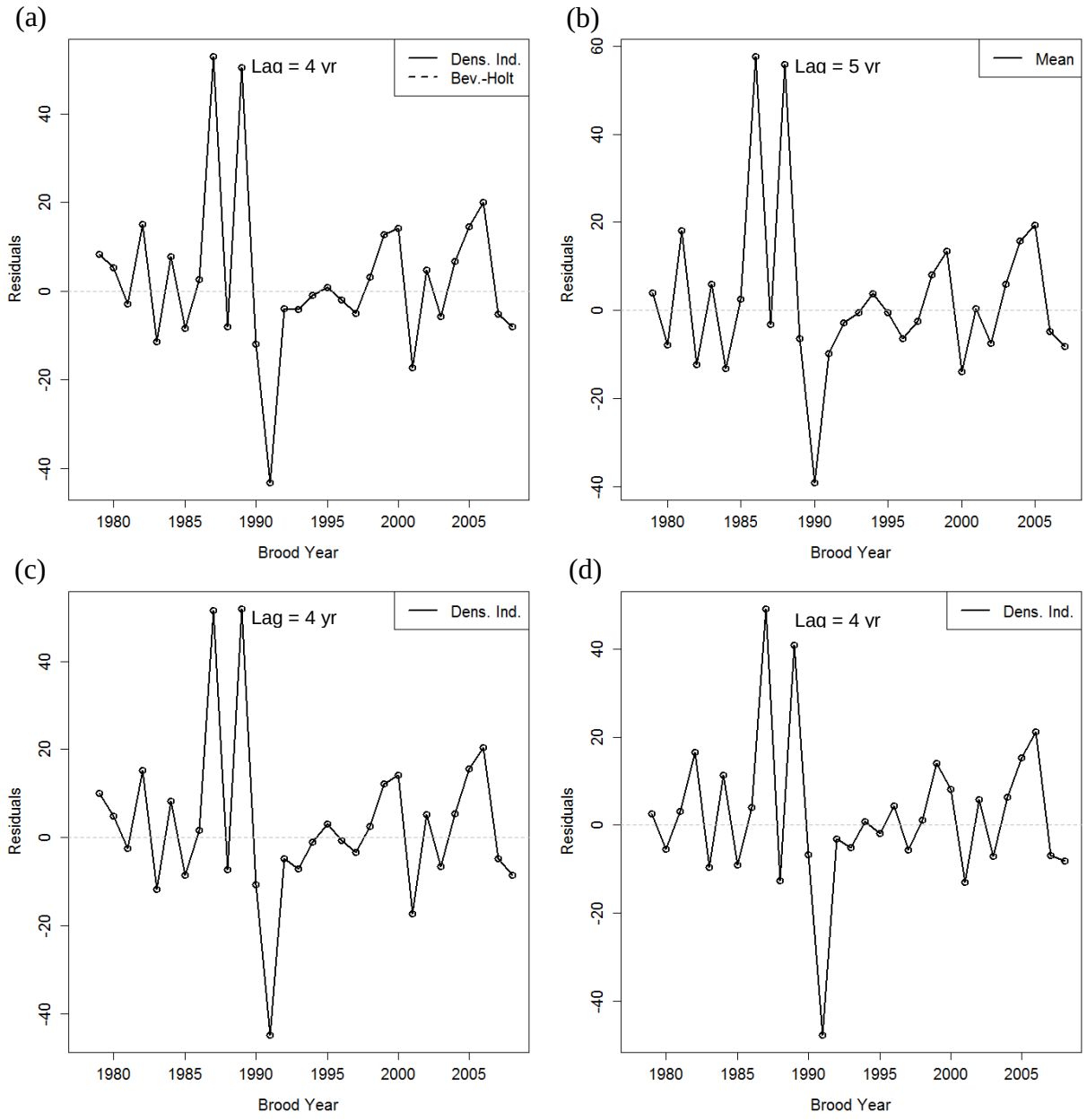

Figure 3.7. Autocorrelation-adjusted time series of stock recruitment residuals by brood year (1978-2008) of competitive models ($\Delta AIC_c < 2$) fitted to relationships (Fig. 3.6) between reproductive potential and recruitment for eastern Bering Sea snow crab. Recruitment is indexed as the area-swept abundance of instar vii juveniles (25-33 mm CW) and reproductive potential estimated as the area-swept abundance of (a-b) (b) adult male, (c) mature male, (d) mature female; and (e) summer egg production index, (f-g) middle domain egg production index, and (h-i) southern middle domain egg production index. Note that the residual plots are the same (panel b) for all indices for which mean recruitment was the only competitive model

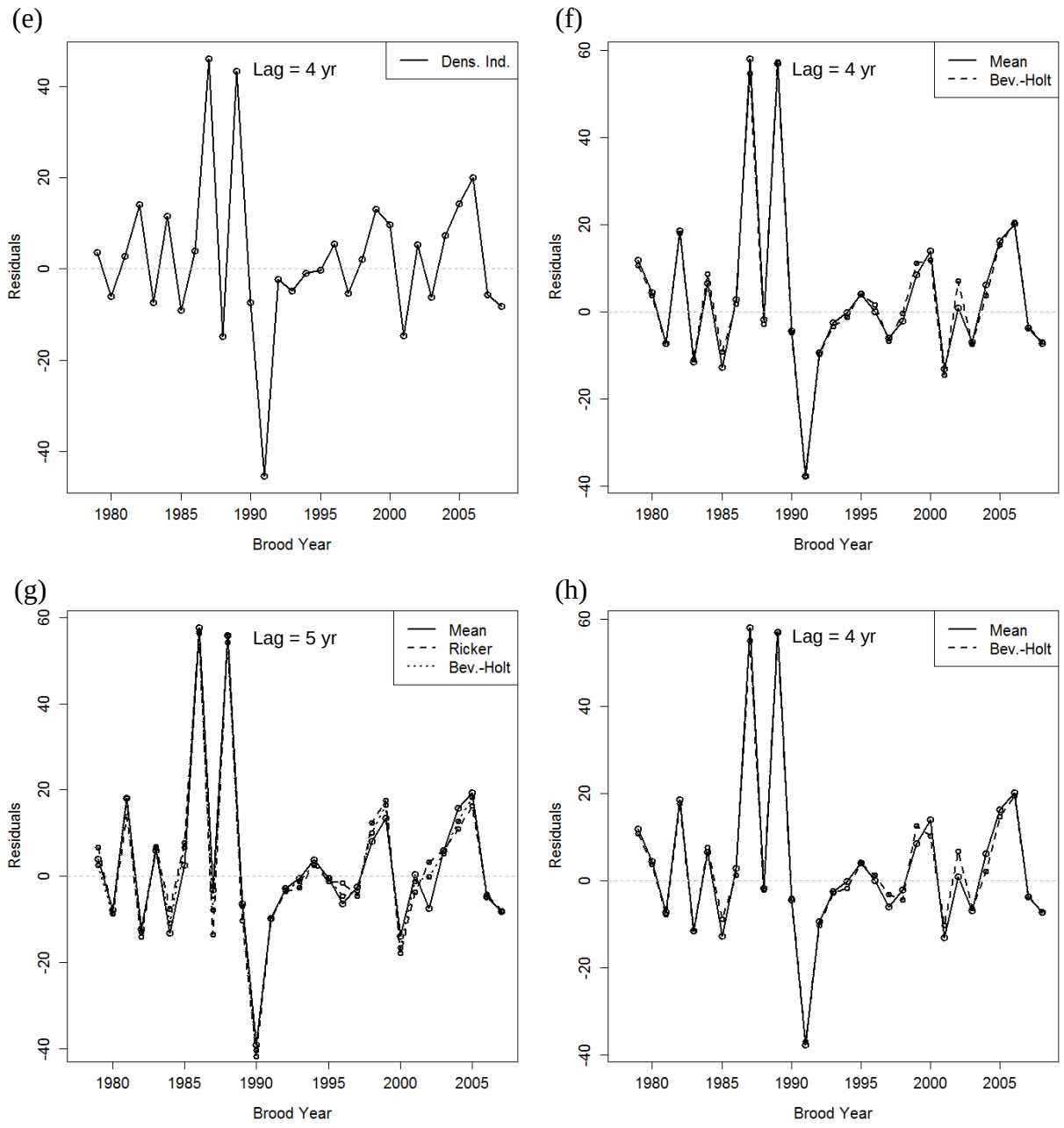

Figure 3.7. Continued

(i)

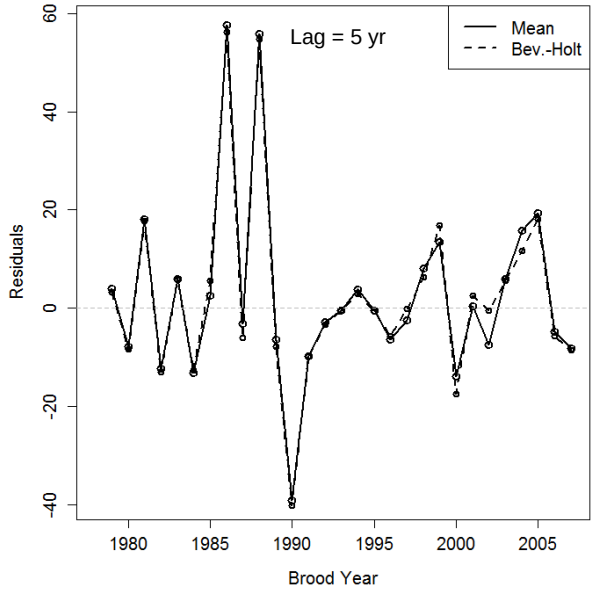

Figure 3.7. Continued

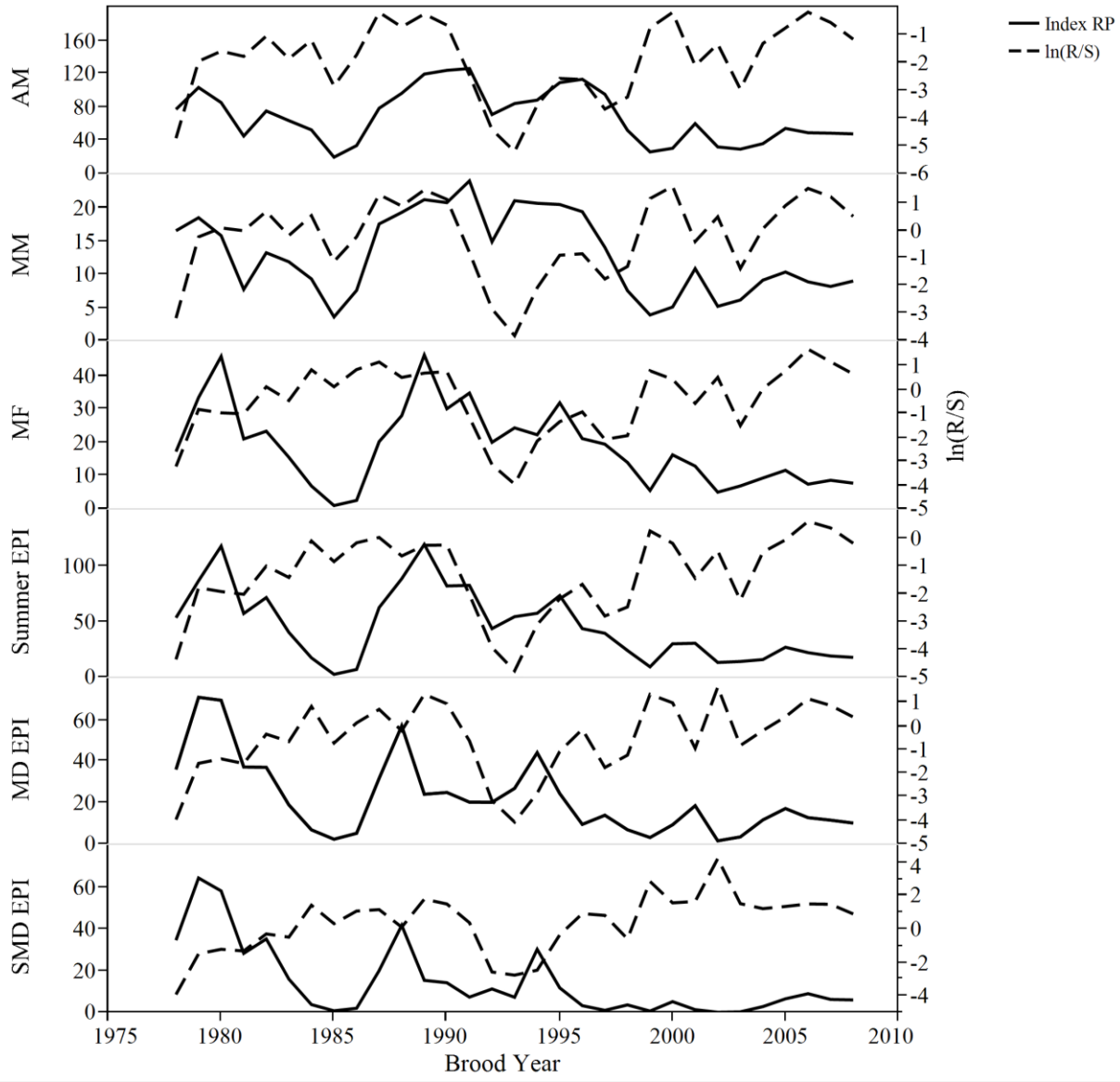

Figure 3.8. Indices of reproductive potential and stock productivity for snow crab in the eastern Bering Sea by brood year at a lag of 4 years from oviposition/fertilization to recruitment. Indices of reproductive potential (RP, left y-axis, solid line) are adult male (AM), mature male (MM), mature female (MF), summer egg production index (Summer EPI), egg production in the Middle Domain (MD EPI), and egg production in the southern middle domain (SMD EPI) and the recruitment index was estimated as the area-swept abundance of instar vii juveniles (25-33 mm CW). Stock productivity was estimated as the natural logarithm of recruits per unit of reproductive potential (ln(R/S), right y-axis, broken line).

Appendix 3.A

Modeling Abundance by Shell Condition and Proportion Biennial

Development of an index of annual egg production necessitated the development of a method to estimate the proportion of females not expected to undergo oviposition within a year due to a biennial schedule of reproduction associated with cold bottom temperatures. To do so, first, indices of mature female abundance were calculated from the trawl survey database (Zheng and Kruse, 2014). Area-swept abundance estimates are considered indices because they are not corrected for known variation in catchability of EBS snow crab by sex, CW, and bottom type (Somerton et al. 2013). Second, a stage-based model (Fig. 3.2a), structured by female shell condition, was developed to estimate survival and transition rates between shell condition classes. Females of advanced shell condition, SC4 and SC5, were combined into a plus group (SC4+), hereafter referred to as very oldshell. The stage transition matrix (**M**) was defined as:

$$\begin{bmatrix} n_n(t+1) \\ n_o(t+1) \\ n_v(t+1) \end{bmatrix} = \begin{bmatrix} P_n & 0 & 0 \\ G_{no} & P_o & 0 \\ 0 & G_{ov} & P_v \end{bmatrix} \begin{bmatrix} n_n(t) \\ n_o(t) \\ n_v(t) \end{bmatrix} \quad \text{(A.1)}$$

where n_i *(i = n, o, v)* is the abundance of newshell (*n*), oldshell (*o*), or plus group (very oldshell, *v*) females. P_i (*i = n, o, v*) and G_x (*x = no, ov*) are the proportion of females staying at that stage or transitioning to the subsequent stage, respectively. Model indices of annual abundance were estimated as:

$$\begin{aligned} \hat{n}_n(t+1) &= \hat{n}_n(t)e^{v} + \hat{S}(\hat{n}_n(t)\hat{P}_n) \\ \hat{n}_o(t+1) &= \hat{S}(\hat{n}_n(t)(1-\hat{P}_n) + \hat{n}_o(t)\hat{P}_o) \quad \text{(A.2)} \\ \hat{n}_v(t+1) &= \hat{S}(\hat{n}_o(t)(1-\hat{P}_o) + \hat{n}_v(t)) \end{aligned}$$

where $\hat{S}$ is the constant survival rate; e^{r_t} is log-normally distributed recruitment to the newshell group each year from 1978 to 2012; $\hat{n}_i$is the model estimated abundance for newshell, oldshell, and plus group females; and $\hat{P}_i$ is the model-estimated proportion of females staying within a

shell condition class in subsequent years. Both $\hat{S}$ and e^{r_t} were estimated within the model. The initial abundance ($N_{0,x}$) for each stage was also estimated within the model. The model was fitted by minimization of residual sum of squares (RSS):

$$we^{r_t} + \sum_t \left(ln(A_{i,t} + 0.0001) - ln(\hat{A}_{i,t} + 0.0001) \right)^2, \qquad \text{(A.3)}$$

where $r = N(0,\sigma^2)$; $i=n,o,v$; t=1979-2012; $A_{i,t}$ is the trawl survey area swept abundance; and $\hat{A}_{i,t}$ is the model estimate of abundance. The sensitivity of model parameter estimates to possible changes in the underlying data was evaluated by fitting the model to portions of the time series associated with changes in survey gear (1982-2012) and spatial extent of the survey area (1989-2012) versus the entire time-series (1978-2012). The optimal value of the penalty parameter (w), which constrained variability in annual recruitment, was determined by iteration from 0.001 to 10 and evaluated against the ΔRSS at each step. A value of w=1.0 was chosen for the final model. The sensitivity of the final model to changes in individual parameters was examined by setting each to the upper and lower confidence bounds while holding all other parameters at estimated values.

Biennial Reproduction

Females completing the molt to maturity and undergoing oviposition in the winter/spring, observed as newshell in the summer survey in year t, should have embryos in the early stages of development. Thus, females on a biennial reproductive cycle, triggered by an extended period of diapause early in embryo development, would be observed as oldshell females brooding egg clutches in the intermediate stages of development during the summer of the following year ($t+1$). This group of females will be referred to as "reproducing biennially" hereafter. The abundance of females likely to reproduce biennially was inferred by estimation of the proportion of the area-swept abundance index observed at survey stations below threshold bottom temperatures ($P_{i,t}^{BT<x}$) for each year where x = 0, 0.5, and 1.0 °C. After establishing initial conditions, the proportion of females reproducing biennially (P_i^B) was iteratively estimated by year (t) for shell condition classes o and v as:

$$P_{o,t}^{B} = \frac{\hat{S}\left(n_{n,t-1} \cdot \hat{P}_{n,t-1}^{BT<x}\right) + \hat{P}_{o} \cdot \left(n_{o,t-1} - A_{o,t-1}^{B}\right) \cdot P_{o,t-1}^{BT<x}}{n_{o,t}},$$

(A.4)

$$P_{v,t}^{B} = \frac{\hat{S}\left(\hat{G}_{o,v} \cdot \left(n_{o,t-1} - A_{o,t-1}^{B}\right) \cdot P_{o,t-1}^{BT<x} + \left(n_{v,t-1} - A_{o,t-1}^{B}\right)\right)}{n_{v,t}}$$

where $A_{o,t-1}^{B}$ and $A_{v,t-1}^{B}$ are the product of the trawl survey area-swept abundances and the proportion of oldshell and very oldshell females reproducing biennially in the prior year and $\hat{G}_{o,v}$ is the model-estimated transition rate between the oldshell and plus group classes in subsequent years.

General Conclusions

Chapter 1 of this dissertation provided preliminary evidence that high harvest rates of large males and female-skewed sex ratios are associated with reduced female sperm reserves among six stocks of Tanner crab in Southeast Alaska. Egg viability and fecundity analyses also demonstrated that substantial reductions in female egg production due to sperm limitation were unlikely. Differences in sperm reserves among females of varying shell condition, measured as both spermathecal load and sperm cell counts, revealed important relationships informative of mating dynamics and were reinforced by observations of variability in the prevalence of qualitative indicators of recent mating. These patterns suggested that mating of hard-shell, multiparous females is commonplace but that females are not acquiring usable sperm reserves greatly in excess of those required for fertilization of one additional clutch through ontogeny. The presence of fresh ejaculate, evidenced by a white-layer at the ventral end of the spermathecae (Duluc et al. 2005), was a definitive indicator of increased sperm reserves or resilience to a lack of mating during the proximal mating period for both primiparous and multiparous females. The size-fecundity relationship of Tanner crab in Southeast Alaska was similar to that of other Tanner crab stocks in Alaska (Hilsinger 1976, Somerton and Meyers 1983), suggesting that this may be a conservative biological trait for this species.

The implications of this study are valuable for several purposes related to fishery management. Direct monitoring of female sperm reserves and egg viability provided easily interpretable information on the risk of recruitment overfishing due to sperm limitation. Second, while these results will be strengthened by additional years of monitoring, our results suggest that gradients of sex ratio and exploitation rate derived from pot survey catch-per-unit-effort can be used to identify stocks with increased relative risk of sperm limitation. Development of functional relationships between exploitation rate, sex ratio, and female reproductive potential will be a key step in the future development of models which can explicitly account for the effects of male-only harvest on female reproductive success (e.g. Alonzo and Mangel 2004). Concurrent monitoring of female sperm reserves among multiple discrete stocks with contrasting states of sex ratio and exploitation rate may be an effective means of accomplishing this task in a shorter time period that could result from long-term monitoring of a single stock. Finally, the presence

of fresh ejaculate is a robust indicator of differences in female sperm reserves and would be an approach easily deployed during fishery independent surveys to efficiently evaluate female sperm reserves for stocks where fishery managers have concerns about potential sperm limitation.

Investigation of variability in female fecundity and embryo quality in Chapter 2 provided new insights into the primary factors influencing the reproductive potential for female snow crab in the EBS. Fecundity increased with female size and was similar among primipara and young multipara. Fecundity decreased among older multipara likely due to senescence. A striking attribute of the size-fecundity relationship was high variance at size for both young and old multipara in comparison to primipara. Hypothesized mechanisms to explain these differences included contrasting mating dynamics, duration of ovarian maturation, and potential variability in factors influencing maternal condition due to spatial segregation of the two groups into areas with differing environmental and benthic productivity regimes (Alunno-Bruscia and Sainte-Marie 1998, Ernst et al. 2005). Intriguingly, female fecundity declined ~10% in multiparous females that lacked fresh ejaculate, an indicator of recent mating. However, reduced fecundity may not be directly attributable to sperm limitation due to the confounding factors of varying chronological age relative to the terminal molt within shell condition classes (Ernst et al. 2005, Fonseca et al. 2008), onset of the effects of senescence, and potential changes in attractiveness as mates with ontogeny (Sainte-Marie et al. 2008). Similarity of size-fecundity relationships for females with early versus late in embryo development indicated that significant embryo loss during brooding was unlikely.

Embryo quality did not vary markedly with maternal characteristics for female snow crab. Instead, a weak association between increased fecundity and an index of embryo lipid content suggested that gradients in maternal condition may be associated with changes in fecundity rather than embryo quality regardless of shell condition. Findings from our study also suggested that EBS snow crab have lower fecundity-at-size than conspecifics from the Atlantic Canada and Japan. Hypotheses to explain potential differences are not immediately apparent, but empirical comparison of size-fecundity relationships among stocks could advance understanding of possible among stock variation in reproductive potential. Taken together, these results suggested that female fecundity is a product of interacting endogenous (maternal size, condition, and age)

and exogenous (mating success and habitat quality) factors which vary with mature female ontogeny, indexed by shell condition.

Indices of female egg production were developed as refined indices of female reproductive potential in Chapter 3. These indices advanced our understanding of the role of reproductive potential on stock renewal dynamics for EBS snow crab. Due to the absence of empirical data, a stage-based model structured by shell condition and time series of female distribution and abundance by bottom temperature was developed to infer the impact of biennial reproduction on egg production. However, the fraction of females inferred to reproduce based on exposure to cold near-bottom temperatures was clearly biased likely due to discrepancies between model estimates and the observed data. Despite this limitation incorporation of increasing levels of information (demography, size-fecundity, and clutch fullness index) resulted in reductions in estimated egg production compared to simpler indices. Recruitment estimated from refined size/age-at-instar information (Ernst et al. 2012), was highly variable and cyclical. Historically low levels of recruitment preceded the decline in EBS snow crab abundance and harvest observed in the late 1990s. Positive functional relationships between reproductive potential and recruitment were identified but were primarily driven by the choice of lag between reproduction and recruitment. Patterns of stock productivity and recruitment deviations suggest a switch from favorable to unfavorable conditions for formation of strong cohorts between the late 1980s and mid 1990s. Further characterization of stock dynamics in relation to likely environmental determinants of recruitment success during this period may provide unique insight into factors impacting stock productivity and future harvest.

Continued research progress, focused on relationships between mating dynamics of male snow crab and female sperm reserves, will be necessary for the estimation of biological reference points based on relationships between male fishing mortality and threshold levels of fertilized egg production for EBS snow crab (NPFMC 2011). In the meantime, fishery management continues to use biological reference points derived for males only. Currently, published empirical data are lacking on sex ratios *in situ* and the characteristics of males (e.g. adolescent versus adult) mating with pubescent, primiparous, or multiparous females in the EBS. Primparous female sperm reserves vary cyclically with fluctuating sex ratio for snow crab in Atlantic Canada (Sainte-Marie et al. 2002), but sperm reserves of primiparous females are much

lower in the EBS than in Atlantic Canada and lack strong cyclic variation (Slater et al. 2010, L. Slater, pers. comm. ADF&G, Kodiak, AK), suggesting that factors determining sperm reserves may differ between systems.

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